Title: Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment (FOR*Ma*T)

NCT #: NCT04310930

Document Type: Study Protocol

Document Date: 20 April 2022

MASTER PROTOCOL

Protocol Version	Version 3.7
Protocol Date	20 April 2022
Protocol Number	FOR*Ma*T001
Funding	1- Medical Research Future Fund, Australian Government Department of Health 2- Cystic Fibrosis Foundation 3- Anonymous Donor 4- Thoracic Society of Australia and New Zealand 5- The University of Queensland 6- Children's Hospital Foundation
Australian and New Zealand Clinical Trials Registry Number	ACTRN12618001831279
ClinicalTrials.gov Identifier	NCT04310930
Sponsor	The University of Queensland

CONFIDENTIALITY STATEMENT
This document is confidential and is to be distributed for review only to Investigators, potential Investigators, study staff and applicable Ethics Committees. It is understood that this information will not be disclosed to others without written authorisation from the Sponsor except to the extent necessary to obtain informed consent from those persons to whom the investigational product may be administered.

TABLE OF CONTENTS

TABLE OF FIGURES

ABBREVIATIONS

ADL	Activities of Daily Living
AFB	Acid-Fast Bacilli
ATS	American Thoracic Society
AUC	Area Under the Curve
aSPV	Average Slow Phase Velocity
BAL	Bronchoalveolar Lavage
BAR	Bayesian Adaptive Randomisation
BCM	Biased Coin Minimisation
BTS	British Thoracic Society
CART	Correlation and Regression Tree
CCA	Canonical Correspondence Analysis
CCHR	Centre for Children's Health Research
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CHU9D	Child Health Utility 9D
CM	Central memory
Cmax	Maximum serum concentration
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DSC	Drug Selection Committee
DST	Drug Susceptibility Testing
DVA	Dynamic Visual Acuity

ECG	Electrocardiogram
eCRF	electronic Case Report Form
EM	Effector Memory
EMRA	Effector Memory RA+
ETDRS	Early Treatment and Detection of Diabetic Retinopathy
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in one second
FOR*Ma*T	Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment
GCP	Good Clinical Practice
GLI	Global Lung Initiative
GPLs	Glycopeptidolipids
HIT	Head Impulse Test
HRCT	High-Resolution Computed Tomography
HREC	Human Research Ethics Committee
HRQoL	Health Related Quality of Life
ICH-GCP	International Council for Harmonisation Good Clinical Practice Guidelines
ID	Identification
iDSMB	independent Data Safety Monitoring Board
IV	Intravenous
LAI	Liposomal Amikacin for Inhalation
LLOQ	Lower Limit of Quantification
MABS	*Mycobacterium abscessus*
MABS-PD	MABS Pulmonary Disease
MABS-R	Rough MABS
MAC	*Mycobacterium avium* Complex
MCRI	Murdoch Children's Research Institute

MDR-TB	Multi Drug Resistant-Tuberculosis
MIC	Minimum Inhibitory Concentration
MRL	Mycobacterial Reference Laboratory
NTM	Non-Tuberculous Mycobacteria
PBMCs	Peripheral Blood Mononuclear Cells
PCA	principal component analysis
PedsQL™	Pediatric Quality of Life Inventory
PI	Principle Investigator
PICF	Participant Information and Consent Form
PK	Pharmacokinetics
PPD	Purified Protein Derivative
PPI	Patient and Public involvement
QALY's	Quality-Life Adjusted Years
QoL	Quality of Life
R	Rough
RCT	Randomised Control Trial
RDA	Redundancy Analysis
REMAP-CAP	Randomized, Embedded, Multifactorial Adaptive Platform trial for Community-Acquired Pneumonia
ROS	Reactive Oxygen Species
rRNA	ribosomal ribonucleic acid
SAEs	Serious Adverse Events
SAHMRI	South Australian Health and Medical Research Institute
SF-36	Short form-36
S	Smooth
SIV	Site Initiation Visit

SNHL	Sensorineural Hearing Loss
SOP	Standard Operating Procedure
TB	Tuberculosis
TDM	Therapeutic Drug Monitoring
TLRs	Toll Like Receptors
TSC	Trial Steering Committee
USMs	Urgent Safety Matters
VAS	Visual Analogue Scale
vHIT	Video Head Impulse Test
VNG	Video-Nystagmography
VOR	Vestibulo-ocular Reflex
VRBT	Vestibular Rehabilitation and Balance Therapy
WGS	Whole Genome Sequencing

CHIEF INVESTIGATORS STATEMENT

I confirm that I have read the FOR*Ma*T Master Protocol **Version 3.7, Date 20 April 2022**. As the Chief Investigator, I understand it, and I agree to adhere to the study conduct requirements and agree to conduct this protocol in accordance with International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), the Declaration of Helsinki, the United States (US) Food and Drug Administration (FDA), and local regulations and guidelines. I agree to report all information or data in accordance with the protocol, and in particular I agree to report any serious adverse events. I will accept the monitors', auditors' and regulatory inspectors' oversight of the study. I will promptly submit the protocol to the applicable ethical review board as required.

…….....

Chief Investigator Signature

……..

Chief Investigators Name

……..

Date (dd/mmm/yyyy)

1 INTRODUCTION

1.1 SYNOPSIS

Background: Mycobacteria in the *Mycobacterium abscessus* group (MABS) are a species of non-tuberculous mycobacteria (NTM) found in water and soil habitats that exhibit high levels of intrinsic multi-drug resistance (1). NTM includes more than 160 species that are recognised opportunistic human pathogens with chronic pulmonary infections the most common clinical presentation. Individuals with underlying inflammatory lung diseases are more susceptible to MABS pulmonary disease (MABS-PD), but MABS also affects patients with no underlying condition. MABS-PD can result in significant morbidity, increased healthcare utilisation, accelerated lung function decline, impaired quality of life (QoL) (2), more challenging lung transplantation (3), and increased mortality (2). Of particular concern is the increasing prevalence of pulmonary infections occurring worldwide in patients with bronchiectasis and cystic fibrosis (CF), with prevalence between 5 to 20%. (2, 4, 5). There is real evidence of this in Queensland, Australia (where the infection is notifiable) with the prevalence increasing from 0.85/100,000 in 2001 to 2.35/100,000 in 2016, where now over 100 cases are reported annually, clearly illustrating the emerging threat of this infection. While the increasing prevalence might be reflective of enhanced surveillance and improved microbiological detection (6-8) (9) the reasons for this changing epidemiology are poorly understood (2, 4, 10, 11).

Treatment regimens for MABS are highly variable, not evidence-based and involve complex, expensive and often poorly tolerated drug combinations for prolonged periods (>12 months). Some individuals will have positive cultures that clear spontaneously, some will initially have positive cultures without obvious MABS-PD but go on to develop disease at a later stage, and some may present with established MABS-PD. MABS-PD can be associated with a rapid decline in health status, and there is evidence that successfully clearing infection is associated with better health outcomes (12, 13). However, toxicity, poor tolerance and the prolonged and complex nature of therapy may increase the reluctance of clinicians to initiate treatment and for patients to accept it. A recent systematic review and meta-analysis revealed that such treatment regimens are often ineffective and may even worsen QoL (14). The costs and treatment burden of NTM infection are high, and highest for MABS-PD, estimated at $AUD12-28,000/month (15) highlighting the need to assess the healthcare costs and cost-effectiveness of therapies to inform health policy around NTM. Pathogen, host and treatment factors all likely play a role in clinical and microbiological outcomes.

Aims:

1. To build an iterative, standing, platform trial with innovative and adaptive properties to evaluate combinations of therapies for patients with MABS-PD. Initially this will test therapies that are currently used and recommended in published international consensus guidelines and are the basis for the current treatment guidelines for MABS-PD. Once the best combinations have been

established the platform described in the Master Protocol will have the capacity to add new treatments and to eliminate therapies because of futility as they either lack efficacy or cause unacceptable toxicity. The data obtained as part of the trial will be used to plan for new waves of the platform trial using novel therapeutic approaches that may be tested against the previously determined optimal approaches, thus leading in an iterative fashion to improving microbiological clearance and health outcomes associated with MABS-PD.

2. To use the opportunities afforded by the clinical trial platform to establish discovery studies to:
    a. Understand the effects of MABS-PD and therapeutic interventions on health-related quality of life and determine the cost effectiveness of proposed therapy combinations;
    b. To develop strategies for optimising drug dosing using robust pharmacokinetics;
    c. Understand susceptibility to MABS-PD and develop biomarkers of clinical disease, disease progression and response to therapy;
    d. Investigate the genomics of human MABS strains and antibiotic resistance genes and impact of therapeutic interventions.
3. To investigate the use of registries to facilitate the long-term monitoring of patient outcomes from MABS-PD and treatment.

Methodology: Entry into the Finding the Optimal Regimen for *Mycobacterium abscessus* Treatment (FOR*Ma*T) trial can occur at two different levels; 1- participants of any age from their first MABS isolate and not receiving current MABS therapy are eligible to enrol in the observational cohort and 2- participants of any age meeting the American Thoracic Society (ATS) criteria for the diagnosis of MABS-PD and are untreated for MABS-PD are eligible to enrol in the intervention program (the trial). Participants initially enrolled into the observational cohort who go on to meet the ATS criteria for MABS-PD can transition to the intervention program at any time. Intervention program participants will be randomised to receive MABS-PD therapy combinations and additional outcomes will be assessed.

Participants in both the observation cohort and intervention programs will contribute the same core data, thus providing the opportunity to examine what happens to both treated and untreated patients with positive cultures longitudinally as well as the transition to MABS-PD. The primary outcome of the intervention program is microbiological clearance of MABS taking into account the toxicity of the interventions.

The intervention program (trial) within the FOR*Ma*T platform will adopt an adaptive design with Bayesian adaptive randomisation (BAR) where there is randomisation across three or more interventions and may also include interim rules for dropping or adding treatment arms. Our approach will carry forward the most promising arms that achieve a minimum level of efficacy with acceptable toxicity, known as the "All Promising" approach. If a treatment is carried forward until the end it will

be tested against control/reference group and recommended if the test statistic is above a certain threshold. The intervention program will consist of sequential Phase II trials, including a randomised intensive treatment phase, followed by a randomised consolidation treatment phase. In the future, interventions reaching a probability threshold of demonstrating success at the end of the Phase II trial will have the potential to seamlessly continue to recruit to a Phase III study to enable the Phase II data to be utilised as part of Phase III study, thus reducing the resources required to achieve high quality evidence on which to base treatments.

The FOR*Ma*T Master Protocol describes the adaptive platform trial, to evaluate microbiological, functional, radiological and quality of life outcomes of interventions utilised in the treatment of MABS-PD, in combination with the observational cohort.

1.2 PROTOCOL STRUCTURE

The FOR*Ma*T trial is designed as a standing iterative, platform trial with innovative and adaptive properties, conducted alongside an observational cohort who will receive the same follow-up as trial participants. This is reflected in the multi component structure of the FOR*Ma*T Master Protocol outlined in Figure 1. The framework for the FOR*Ma*T trial and observational cohort is established within the FOR*Ma*T Master Protocol. The interventions with statistical methodologies, discovery studies, registry interactions, and the documents and standard operating procedures (SOPs) related to the trial are described in specific Appendices.

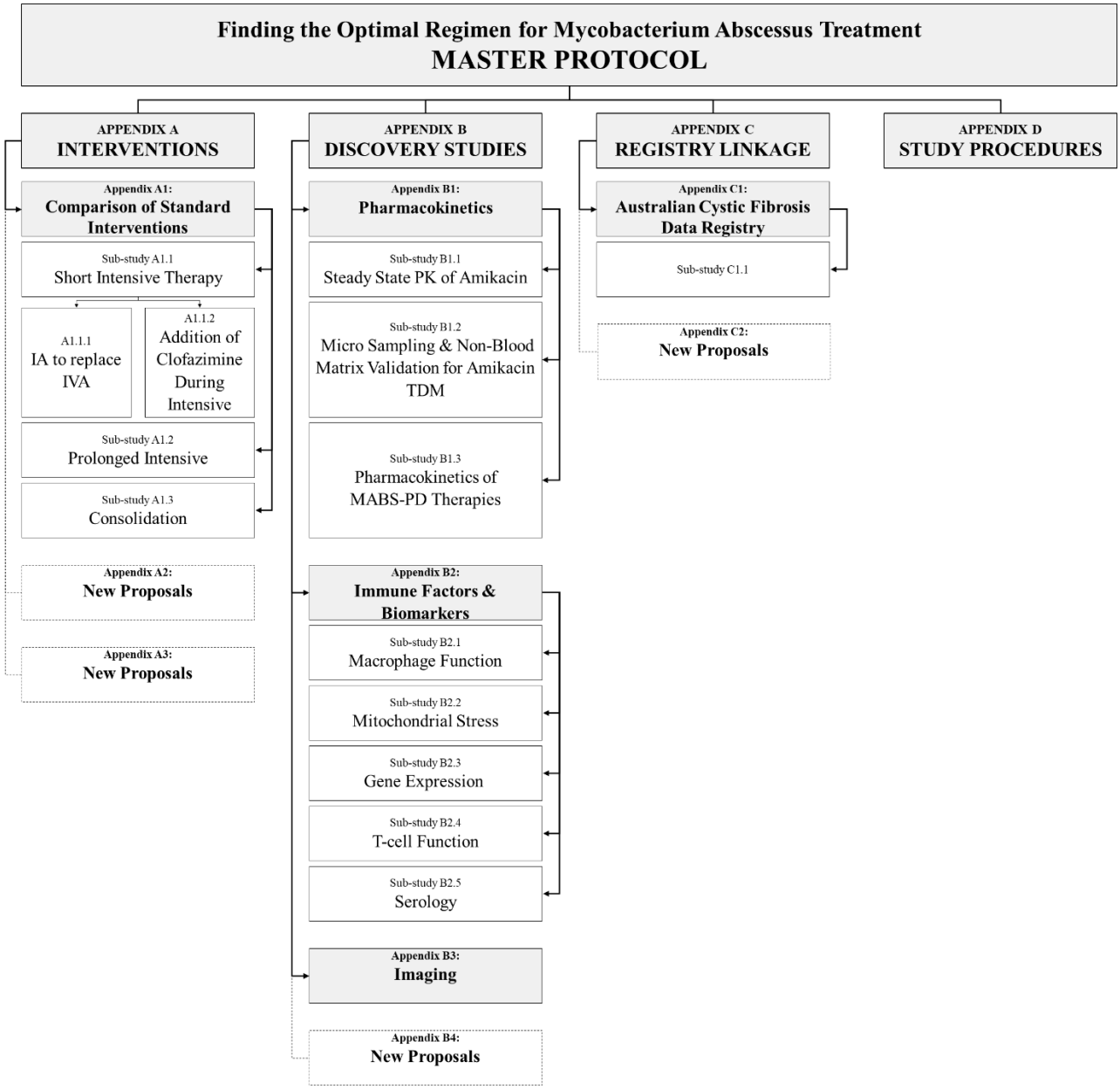

Figure 1: FOR*Ma*T Master Protocol design

1.3 FOR*Ma*T Master Protocol

The FOR*Ma*T Master Protocol outlines the core structure, procedures and processes of the FOR*Ma*T trial. The information described in the FOR*Ma*T Master Protocol applies to all trial sites and participants regardless of if they are receiving intervention treatment for MABS-PD or they are enrolled in the observational cohort, unless otherwise stated.

The Master Protocol has the following structure:

- The background and rationale for determining the optimal treatment regimens for MABS-PD.

- The objectives of the FOR*Ma*T trial (including both the intervention trials and the observational cohort).
- The design of the FOR*Ma*T trial including the two-level approach to assessing eligibility into the trial, trial endpoints and the statistical methodology to test multiple research objectives.
- The FOR*Ma*T trial conduct detailing trial timelines, recruitment methods and consent processes, the core trial procedures and schedule of assessments, safety monitoring processes and data management procedures.
- The FOR*Ma*T trial oversight, ethical and administrative considerations are outlined.

1.3.1 FOR*Ma*T Appendices

The FOR*Ma*T Appendices describe in detail, the information specific to the Appendices and the sub-studies nested within them. As such, Appendix specific information is not described within the FOR*Ma*T Master Protocol but rather, the Master Protocol sets the framework within which the Appendices exist. As the trial progresses, new interventions and methodologies can be added to the FOR*Ma*T trial through the addition of a new Appendix. Conversely, as interventions and methodologies are found to be futile the corresponding sub-studies can be removed. It is not anticipated that these changes will affect the framework of the Master Protocol. Any changes to Appendix specific sub-studies require ethics approval.

Appendix A-C are structured as;

- Each Appendix has a theme; Appendix A for Interventions, Appendix B for Discovery Studies, Appendix C for Registry Interactions.
- Appendix A describes each intervention programs including intensive and consolidation interventions (figure 2). Appendix A1, describes the first of such intervention programs and if new interventions are added to either the intensive or consolidation phases these would be added in new Appendices A2, A3 etc. Data from previous programs using the same intervention combinations may be incorporated in the analysis of a new program. Thus, data from Appendix A1 could be combined with data from Appendix A2 for example.
- Nested within each of the Appendices are Appendix specific sub-studies; Sub-study A1.1, sub-study B1.1 etc each designed to investigate an objective(s).

The components described within each Appendix are variable and dependant on the nature of the Appendix and the sub-studies nested within it. Overall, FOR*Ma*T Appendices A, B and C will contain the following components:

- The overall objective specific to that Appendix.
- Where relevant, information about the features of the interventions to be investigated.
- Appendix specific eligibility criteria.

- Appendix specific consent requirements.
- Appendix specific procedures to be assessed in addition to those described in the Master Protocol.
- Appendix specific statistical methods and simulations where relevant.

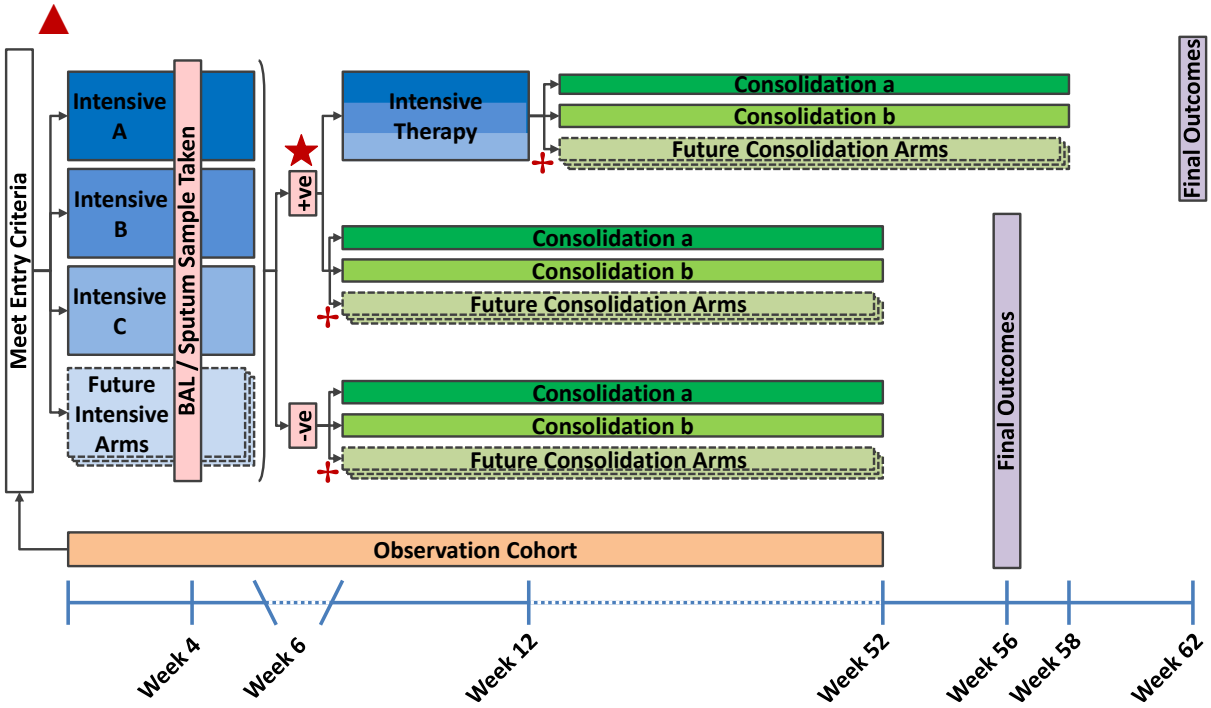

Figure 2: Intervention program participant flow diagram. Eligibility into the intervention program is determined at screening. At randomisation 1 (▲); participants are randomised between the different intensive therapy arms (Intensive A, Intensive B and Intensive C) for a period of 6 weeks. At the end of intensive therapy, it will be determined if participants are still MABS positive, or MABS negative (cleared). Randomisation 2 (★) will ONLY be for participants who are still MABS positive at week six and will allocate participants to either 1) continue intensive therapy or 2) immediately commence consolidation therapy. Randomisation 3 (✢) allocates participants to the consolidation therapy arms either at week 6 or at week 12 depending on MABS clearance at the end of week 6 and randomisation 2 (if relevant). This figure demonstrates the capacity to add future intensive and consolidation arms.

Appendix D;

- Appendix D outlines the documents and procedures related to the trial. This includes the master templates for the child/adolescent information sheet and the participant information sheet, the parent/guardian consent form and the participant consent forms, appendix specific consent and information forms and the HRQoL and utility questionnaires. All of the trial procedures and the sample processing and transport requirements for the Appendices are outline in Appendix D.

2 BACKGROUND

2.1 Microbiology of MABS

Of the many pathogenic NTM species, MABS are recognised as causing the most serious pulmonary infections, associated with the greatest problems of antibiotic resistance, toxicity, and treatment failure (12, 16). MABS are currently divided into 3 subspecies that include *M. abscessus* subspecies *abscessus* (*M. a. abscessus*), subs. *massiliense* (*M. a. massiliense),* and subs. *bolletii* (*M. a. bolletii)*. While there is variation in the prevalence of the subspecies in different populations, *M. a. abscessus* is the most common overall (45-68%), followed *M. a. massiliense* (20-55%) and *M. a. bolletii* (8-25%) (17-19). Progression of MABS-PD due to the different subspecies (20) appears to be similar, although treatment outcomes vary significantly and are partially explained by differential antimicrobial susceptibility to macrolide antibiotics. MABS are intrinsically drug resistant to multiple classes of antibiotics and they can also acquire antibiotic resistance genes to macrolides and aminoglycosides, leading to clearance rates of ≈50% following intensive therapy (18, 21, 22). After apparently successful treatment, relapse or recurrences with new strains occur in 15-33% of patients (18). Nevertheless, macrolides provide the therapeutic backbone of guideline-based MABS treatment (1, 4). Furthermore, they are the only antibiotic class where there is some correlation between *in vitro* susceptibility data and clinical response (14). Macrolide resistance in MABS is either constitutive or inducible (19). The less common, constitutive resistance may be acquired during macrolide therapy and results from mutations in the 23S rRNA gene (*rrl*). Inducible macrolide resistance is related to the MABS ribosomal methyl transferase gene, *erm(41).* Such isolates appear susceptible at day 3 following infection, but resistant by day 14 using prolonged incubation drug susceptibility testing. *M. a. massiliense* has a truncated and dysfunctional *erm(41)* gene, thus making it more susceptible to macrolides, whereas *M. a. abscessus* and *M. a. bolletii* usually, but not invariably have inducible resistance (19). It is not surprising then that microbiological cure rates of MABS-PD appear partly related to macrolide resistance with clearance rates up to 88% in those with macrolide susceptible isolates, and only 36% in the setting of inducible macrolide resistance (18). Molecular detection of the subspecies, including identifying the *erm(41)* and *rrl* genes is important in understanding treatment response, and potentially targeting novel treatment approaches.

2.2 Epidemiology

NTM can cause both asymptomatic and symptomatic infections in humans (1). Pathogenic strains of MABS have been isolated from potable water, and MABS infections are more prevalent in coastal areas and regions with humid tropical climates (12, 23, 24). While most infections are thought to be acquired from environment aerosols (25), in patients with CF, there is evidence supporting the emergence of worldwide dominant clones (of increased virulence) that may be capable of patient-to-patient transmission (17).

2.2.1 High-risk Patient Populations

CF is an autosomal recessive condition caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Mortality and morbidity of CF patients are predominantly related to chronic suppurative lung disease (26). CF is a risk factor for MABS-PD (and NTM more broadly) and even carrier status of disease-causing mutation in *CFTR* (23) may also increase risk. Other structural lung diseases, bronchiectasis, chronic obstructive pulmonary disease, previous mycobacterial disease (including Tuberculosis (TB) and NTM), severe gastro-oesophageal reflux and immunosuppression, (where dissemination can occur) (11) also increase risk of infection. The age range of CF and non-CF affected patients with MABS-PD overlap, with non-CF patients generally being older than those with CF (>55 vs <30 years) (23).

2.3 Clinical Presentation and Diagnostic Challenges

Due to their ubiquitous nature, the clinical significance of positive MABS cultures in respiratory specimens can be challenging. It may appear transiently in sputum cultures, persistently colonise the lower airways or progress to MABS-PD. The radiological and clinical features of underlying chronic respiratory disorders overlap considerably with changes attributable to MABS-PD making diagnosis and treatment decisions difficult and care is required to follow the ATS criteria in making the diagnosis of MABS-PD. Inclusion into the intervention trial will require meeting all of the ATS criteria including both microbiological and clinical criteria.

2.4 Treatment Regimens Guidelines and Challenges

MABS treatment outcomes differ according to the etiologic organism. Recurrence rates for infection are high, despite successful treatment completion (27) but these approaches have not been evaluated in any trials. Differing treatment outcomes present multiple therapeutic challenges in the treatment of MABS. In recognition of these challenges, the ATS and the United States CF Foundation/European CF Society have published guidelines on NTM pulmonary disease (1, 4). In agreement with the latest Cochrane Review (28), they note there are no drug regimens of proven or predictable efficacy for treating MABS. Therefore, the guidelines are based on expert opinion only and in practice the treatments vary considerably (29). Suggested regimens include an intensive phase of 4-12 weeks (based on microbiological response) of intravenous (IV) antibiotics (usually amikacin, cefoxitin or imipenem + tigecycline) plus an oral macrolide. This is followed by consolidation therapy that includes oral drugs (usually a macrolide, plus others based on antibiograms, tolerability and experience) and an inhaled IV formulation of amikacin for 3 to >12 months. Dosing by individual pharmacokinetic (PK) data and therapeutic drug monitoring (TDM) may lead to optimal drug levels at infection sites and better treatment outcomes, although measuring levels within the lower airways frequently and non-invasively is challenging. Few PK studies involving antibiotics for NTM have been performed and few assays are currently available. Inhaled antibiotics have the potential advantages of achieving higher airway concentrations, while reducing the risk of systemic toxicity. Amikacin for inhalation is not available

commercially and clinicians have used an "off-label" IV form of amikacin (delivered via a nebuliser). Importantly, few randomised controlled trials (RCTs) have been performed for guiding the treatment of MABS-PD. A Phase II RCT (30) of liposomal amikacin for inhalation (LAI) sponsored by Insmed, investigated the safety and efficacy of LAI to treat NTM infection (36% MABS) in addition to highly variable multi-drug therapy in 89 patients with (19%) and without (81%) CF who had failed to clear NTM using previous therapeutic combinations. The trial did not meet the primary endpoint of decreased mycobacterial load, but the treatment group had a higher proportion of subjects with >1 negative sputum (32% vs 9%, P=0.006) and improved 6- minute walk distance compared with standard therapy, suggesting a potential benefit from adding LAI to consolidation therapy. This trial highlighted the difficulties and inefficiency of undertaking conventional clinical trials using the "one population, one drug, one disease" approach with relatively small patient numbers, a heterogeneous population and complex, inconsistent drug combinations. These factors greatly limit the clinical information obtained and contribute to making conventional trials in this patient population difficult to interpret. This contrasts with innovative platform trials, which have advantages for efficiently evaluating multiple treatment combinations (e.g. multi-drug resistant TB (11)) requiring complex drug regimens in a heterogeneous population.

2.5 Health Related Quality of Life

A recent systematic review and meta-analysis revealed that MABS treatments are often ineffective and may even worsen QoL (14). The review strongly recommended that "clinical, functional and QoL parameters should be given more emphasis in the evaluation of treatment outcomes" and that "better applications of current antibiotics are urgently needed" (14, 29). In addition, the costs and the treatment burden of NTM infection are high, and highest for MABS-PD, estimated at $AUD12-28,000/month (15) highlighting the need to assess the healthcare costs and cost-effectiveness of therapies to inform health policy around NTM. Pathogen, host and treatment factors are all likely to play a role in clinical and microbiological outcomes. Consequently, there is an urgent need for evidence to support treatment decision-making for patients with MABS lung infection.

2.6 The Patient Voice

In October 2015, the Food and Drug Administration (FDA) held a public meeting on NTM infection with patients and carers and the key theme to emerge (31), was the need for better, less toxic treatment with lower therapeutic burden. Participants also prioritised "validating and using tools to measure QoL and developing disease specific activity and severity assessment tools" (32). In January 2017, The James Lind Alliance released their top 10 research priorities for people with CF developed by CF patients, their families, and healthcare providers. The 3rd top priority was "What is the best treatment for NTM, including when to start and what medication?" (33). Patients and healthcare providers are asking for evidence to guide the best approaches to manage this challenging infection.

2.7 Generating Evidence Using a Randomised Platform Trial Design

Platform trials using Bayesian statistical models, provide the opportunity to efficiently investigate multiple treatments for difficult-to-treat infections (e.g. multi-drug resistant TB (34)) requiring complex drug regimens in a heterogeneous population and can provide an iterative resource facilitating the translation of finding to improve clinical outcomes (35-37). New adaptive trial approaches are now recognized and accepted by regulatory authorities, including the FDA. Such trials are now being used in infectious diseases (Randomized, Embedded, Multifactorial Adaptive Platform trial for Community-Acquired Pneumonia (REMAP-CAP) (clinicaltrials.gov NCT02735707) and TB (34)) complex chronic diseases (36) and rare oncology conditions (38) MABS-PD is a serious but relatively rare problem which can benefit from such methodology. FOR*Ma*T provides a common platform that will enable a broad enrollment of patients who have and do not have cystic fibrosis and across all ages that enables generalizability but also maintains the ability to examine the heterogeneity of treatment responses across specific subgroups, while also enabling a comparison with non-treated patients through an observational cohort who are followed up simultaneously. The ability to examine novel therapeutic approaches using a common platform, along with the option to seamlessly move from phase II to phase III if warranted, also reduces the resources and the time required to deliver evidence based treatments to patients who need it compared with conducting a series of independent trials.

2.8 Summary

There is no evidence currently to guide therapy for MABS-PD, a complex and increasing health problem. The FOR*Ma*T trial seeks to provide answers to key questions by healthcare providers and patients on the timing and nature of treatments for the growing number of people infected with MABS, as well as the potential to model the progression of this condition in both treated and untreated patients. Furthermore, the trial will provide a platform for improving health outcomes for MABS patients and build a solid foundation for future testing of new therapeutics in the treatment of MABS.

3 OBJECTIVES

3.1 Primary Objective

Determine the optimal therapy for patients in the treatment of MABS-PD.

3.2 Secondary Objectives

1- To investigate the optimal approaches to antibiotic dosing and therapeutic drug monitoring.
2- To investigate the health-related quality of life and cost effectiveness of proposed therapy combinations.
3- Examine the structural changes using lung imaging with chest CT scan associated with the development and progression of MABS-PD, and the effects of treatment combinations and clearance of infection on structural lung disease progression.

4- Compare the change in FEV_1 z-scores measured by spirometry across the treatment combinations.
5- To develop biomarkers to predict the onset of MABS-PD and response to therapies.
6- To understand susceptibility to infection with MABS associated with the development of MABS-PD and host immune responses to infection and with treatment.
7- To characterize the genomics of human MABS strains and antibiotic resistance genes in patients in the observation and intervention studies.

4 TRIAL DESIGN

4.1 Eligibility Criteria

Eligibility criteria for the FOR*Ma*T trial can be applied at two levels:

1- Eligibility into the observational cohort, ***or;***
2- Eligibility into the intervention program.

Potential participants can only be enrolled in the observational cohort or the intervention program at any one time. Provided the eligibility criteria are met, potential participants may either:

1- Enrol directly into the intervention program, ***or;***
2- Enrol into the observational cohort and transition into the intervention program once they satisfy the inclusion criteria for this program which can occur at any time during the trial.

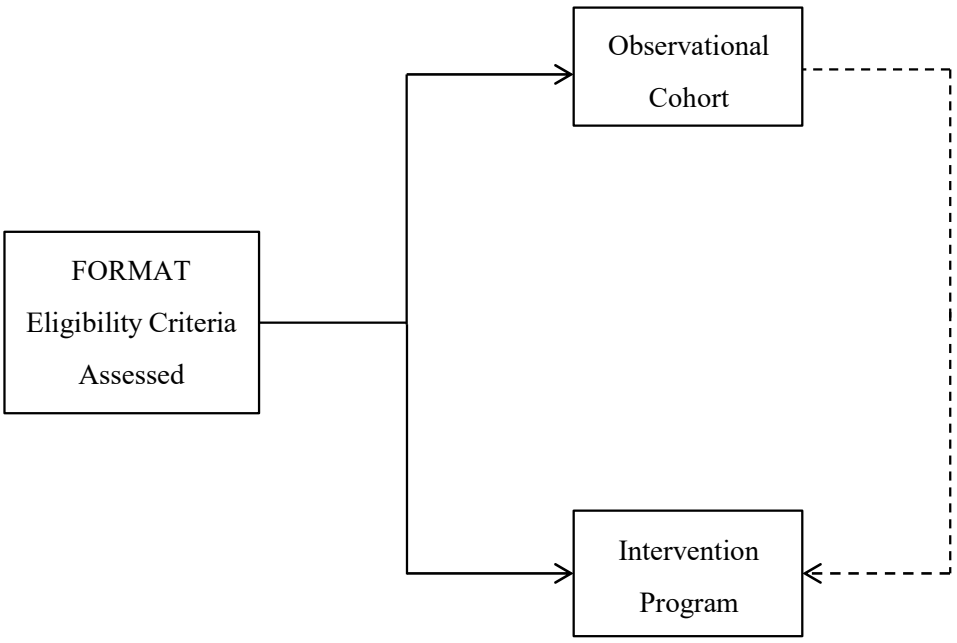

Figure 3: Participant enrolment into the FOR*Ma*T trial. Eligibility into the FOR*Ma*T trial will be assessed at screening. Observational cohort participants who go on to meet the Intervention program eligibility criteria can transition from the observational cohort to the Intervention program.

4.2 Observational Cohort

4.2.1 Observational Cohort Inclusion Criteria

To be eligible to participate in the observational cohort the following criteria must be met:

1- Male and female participants of any age with at least one positive respiratory culture for MABS.
2- Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age.
3- Ability to comply with study visits and study procedures as judged by the site investigator.

4.2.2 Observational Cohort Exclusion criteria

Potential participants will be ineligible to participate in the observational cohort if any of the following criterion are met:

1- Receiving active treatment for MABS within the previous 12 months, except azithromycin for participants with CF and bronchiectasis.

4.3 Intervention Program Eligibility

Potential participants are eligible for the Intervention program (Appendix A) if the criteria below are met. Eligible participants with mixed NTM infections (slow growers + MABS) or with recurrence of MABS infection following completion of previously successful treatment defined as remaining free of positive respiratory cultures for MABS over 12 months post treatment, will be eligible if they met the inclusion and exclusion criteria listed below. For eligible participants with mixed NTM infections additional therapy combinations are available as detailed in section 8 of Appendix A1.

4.3.1 Intervention Programs Inclusion Criteria

1. Positive MABS-PD diagnosis meeting all three American Thoracic Society clinical, radiological and microbiological diagnostic criteria for MABS-PD. Defined as;
   a. ***Clinical:*** Pulmonary symptoms and exclusion of other diagnoses.
   b. ***Radiological:*** Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules.
   c. ***Microbiological:*** MABS positive culture results from at least two separate expectorated sputum samples.

   or

   Positive culture results from at least one bronchial wash or lavage.

   or

   Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli (AFB)) and positive culture for NTM

or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM.

2. Male or female participants of any age.
3. Participant has not received treatment for MABS-PD in the 12 months preceding assessment of eligibility.
4. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age.
5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.

4.3.2 Intervention Program Exclusion Criterion

1- Participants receiving treatment of MABS-PD in the previous 12 months. Use of azithromycin as part of routine treatment for CF and bronchiectasis is not an exclusion.
2- Participants who have a QTc interval of >500 milliseconds.
3- Known hypersensitivity to any of the intervention therapies for which no alternative option(s) have been provided.

4.4 Additional Eligibility Criteria

Appendix specific sub-studies may have additional eligibility criteria which are described in each of the sub-study specific appendices.

Participants with any known or existing drug allergies and/or intolerances to any of the core antibiotics used in any of the intervention treatment arms will still be considered eligible. Alternative drug options are available in the drug dosing tables outlined in Appendix A1 for both intensive and consolidation treatment.

4.5 Trial Endpoints

The primary outcome for the FOR*Ma*T Intervention Trial is MABS clearance from respiratory samples with tolerance. The Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (Refer to Appendix D of FOR*Ma*T Master Protocol) will be applied for the coding of all adverse events (AEs) and serious adverse events (SAEs) that occur during the FOR*Ma*T trial that are categorised as at least "possibly", "probably" or "definitely" related to study medications and/or interactions between study medications and concomitant medications. "Good" tolerance will be defined as no adverse events occurring or only adverse events coded CTCAE grades 1 or 2. "Poor" tolerance will be defined as any adverse events coded as CTCAE grades 3, 4, or 5.

The primary outcome of the FOR*Ma*T Intervention Trial will be assessed at the following timepoints and defined as:

1- For final outcome:
    a. Clearance – four consecutive negative sputum samples (expectorated or induced) collected (≥one week apart) during consolidation treatment and a negative sputum (expectorated or induced) sample culture (collected at least four weeks after completing consolidation therapy,

    or;

    One negative BAL collected at least four weeks after completion of consolidation.
    b. Tolerance – good tolerance of study medications (defined above) from the date of Randomisation 1 to Final Study Visit.
2- For short intensive therapy:
    a. Clearance – three negative sputum samples (induced or expectorated) or one bronchoalveolar lavage (BAL) sample collected at week 4 (culture results received at week 6).
    b. Tolerance – good tolerance of study medications (defined above) from the date of Randomisation 1 to Week 6.
3- For prolonged intensive therapy:
    a. Clearance – three negative sputum samples (induced or expectorated) or one BAL sample collected at week 10 (culture results received at week 12).
    b. Tolerance – good tolerance of study medications (defined above) from the date of Randomisation 1 to Week 12.
4- For consolidation therapy:
    a. Clearance – three consecutive negative sputum samples (expectorated or induced) collected (≥one week apart) during consolidation treatment.

    or;

    One negative BAL collected at least four weeks after completion of consolidation.

    b. Tolerance – good tolerance of study medications (defined above) from the date of Randomisation 3 to Final Study Visit.

Please note: If a positive respiratory sample is reported during any of the above clearance timepoints defined above, then the result(s) will be classified as not achieving MABS clearance.

5 TRIAL CONDUCT

5.1 Site Initiation

The FOR*Ma*T Trial is a multicentre, international clinical trial and the initiation of the FOR*Ma*T Master Protocol and Appendices will be staggered across trial sites. This staggered approach will allow the FOR*Ma*T Trial Management Team to closely examine the effectiveness of the FOR*Ma*T Trial Master Protocol and Appendices. Any changes required to improve the trial processes can be shared and implemented in other trial sites prior to site initiation. Countries participating in the FOR*Ma*T Trial include Australia, Denmark, United Kingdom, Netherlands, New Zealand, France, Ireland and Canada.

5.2 Trial Duration

The FOR*Ma*T Master Protocol describes a standing platform trial with an innovative design tailored to the clinical setting that will enable new therapies to be tested as they become available. As such, there is no limitation to the duration of the FOR*Ma*T trial.

Participants enrolled into either the observational cohort or the intervention program are enrolled for a maximum of 62 weeks. A detailed outline of the total duration of Appendix specific sub-studies is described in the relevant Appendix.

5.3 Recruitment of Potential Participants

Potential participants will be identified at study sites by the treating physician. Once identified, potential participants will be approached by the FOR*Ma*T trial site specific research team or treating physician to discuss the FOR*Ma*T trial and to provide the potential participant with the FOR*Ma*T Participant Information and Consent Form (PICF). FOR*Ma*T trial research team members are required to allow potential participants adequate time to read the PICF and an opportunity to ask any trial related questions and to have these questions answered to the satisfaction of the potential participant.

5.4 Informed Consent

5.4.1 Consent to the FOR*Ma*T Master Protocol

In accordance with the International Council for Harmonisation Good Clinical Practice Guidelines (ICH-GCP) and/or the Declaration of Helsinki, consent to the FOR*Ma*T Master Protocol will be obtained by the FOR*Ma*T trial site research team prior to any study related procedures being performed. All participants, regardless of if they are enrolled in the observational or intervention program are required to consent to the FOR*Ma*T Master Protocol. If the participant is under 18 years of age or unable to provide consent (due to severe cognitive impairment, an intellectual disability, or a mental illness, including patients with dementia) then written consent will be obtained by the participants parent/legal guardian.

The FOR*Ma*T Master Protocol consent form is required to be signed and dated by the individual providing consent or the participant's parent/guardian where appropriate. A copy of the signed consent

form will be given to the participant for their records with the original signed copy stored in the participants file and medical records. Any Independent Review Board (IRB)/ Independent Ethics Committee (IEC)/ Human Research Ethics Committee (HREC) approved changes to the trial Master Protocol will require the participants reconsent.

5.4.2 Consent to Medicare Benefits Schedule (MBS) and Pharmaceutical Benefits Scheme (PBS) Study

Additional consent will be required from participants based at Australian sites only to access:

1- Participant hospital records for the assessment of resource utilisation;
2- Participant Medicare records for a cost-effective assessment in the non-hospitalisation setting of participants treated for MABS-PD;
3- Participant community pharmacy dispensing data.

5.4.3 Consent to Specific Appendix Intervention programs and Discovery studies and Registry involvement

If a participant meets the eligibility criteria for the FOR*Ma*T Appendices and/or specific sub-studies, additional Appendix specific consent will be required in accordance with the procedures detailed above, section 5.4.1. Appendix specific consent requirements are detailed in the Appendices. If additional sub-studies are added or removed from an Appendix, or any changes are made to the Appendix specific sub-studies IRB/IEC/HREC approval for these changes will be sought and will require the participants reconsent to these changes.

5.5 Core Trial Procedures

Core trial procedures refers to the essential trial procedures required for participants who are enrolled in the observational cohort or the Intervention program. Core trial procedures will be measured in accordance with Table 1, schedule of assessments. Additional Appendix specific trial procedure requirements are detailed in the relevant Appendix.

5.5.1 Respiratory Sample for Microbiology Assessment

In accordance with table 1, respiratory samples will be collected as part of eligibility criteria and throughout the study. The collection times will be as per the group enrolled (see sections 5.5.1.5). For screening, the results of a recently collected MABS-positive respiratory sample (collected within six months for all FOR*Ma*T Trial participants and ideally within three months for Intervention Program participants) may be used to meet the eligibility requirements. If the sample is stored, then the sample will be analysed retrospectively once consent to participate in the FOR*Ma*T trial is given. All respiratory samples collected for MABS identification are required to be labelled and sent to local pathology for culture of bacteria including AFB in accordance with local regulations (see Appendix D: SOP for Microbiology Assessment for additional details).

Acceptable methods for collecting a respiratory sample for the microbiological assessment of MABS include:

5.5.1.1 Expectorated Sputum

Participants able to expectorate sputum are required to provide a 1ml expectorated sputum sample in a specimen jar or separate specimen jars if multiple samples are obtained. Specimen jar(s) are to be labelled and sputum samples processed in accordance with site specific pathology requirements for the detection of MABS.

5.5.1.2 Induced Sputum

Sputum induction is indicated for participants incapable of expectorating sputum. Prior to sputum induction a bronchodilator can be administered to minimise bronchospasm. Sputum induction is achieved using 8 mL of 6/7% hypertonic saline nebulised at 5 L/min for 15 minutes. Participants unable to tolerate 6/7% hypertonic saline can use 4.5% hypertonic saline. A 1ml induced sputum sample is to be collected in a specimen jar in accordance with the induced sputum SOP, Appendix D.

5.5.1.3 Bronchoalveolar Lavage

A BAL is indicated if an expectorated or induced sputum sample is unobtainable. Where possible a six-lobe lavage should be collected. If six-lobe lavage is not feasible a minimum two-lobe lavage from the are most affected on chest CT scan should be collected. All BAL samples are to be processed in accordance with site specific procedures.

5.5.1.4 Lung/Airway Biopsy

Lung/airway biopsy is only acceptable for the initial MABS diagnosis, but not as a routine method for MABS microbiological assessment throughout the trial.

Please note, cough swabs are not an acceptable respiratory sampling technique for the FORMaT Trial.

5.5.1.5 Respiratory sample collection time points by enrolling group

5.5.1.5.1 Intervention Program

After screening, Intervention Program participants must provide a respiratory sample(s) at week 4 and week 10 and then monthly sputum sample collections are recommended. If monthly sputum samples are unable to be obtained, then a minimum of four further sputum samples are requested to be collected during consolidation therapy (suggested timepoints: week 18, week 28, week 38 and end of consolidation (week 52 or 58, depending on treatment arm allocation)). A final respiratory sample will be collected four weeks after the end of consolidation therapy at either week 56 or week 62 (determined by the treatment arm participants are allocated). If the participant is unable to produce the minimum sputum samples required during the consolidation phase (weeks 18, 28, 38 and 52/58; in accordance

with the methods described above), then a single BAL sample collected at the final study visit is acceptable to inform final outcome.

5.5.1.5.2 Observational Cohort

After screening, Observational Cohort participants are requested to provide respiratory sample(s) at week 4 and week 10. If the Observational Cohort participant is unable to provide a sputum sample (expectorated or induced) at week 4 or week 10, the one BAL is required to be collected at week 10. Monthly sputum samples are recommended to be collected between Week 14 and Week 52. The final outcome respiratory sample for Observational Cohort participants will be collected at the final study visit (week 56). If the participant is unable to provide the requested sputum samples between Week 10 and Week 52 (in accordance with the methods described above), then a single BAL collected at the final study visit is acceptable to inform final outcome.

5.5.2 Chest Computed Tomography

Chest CT will be performed at screening, week 12 (optional and participant must provide additional consent, see Appendix B3: Imaging) and at the final outcome (week 56 or week 62 depending on treatment arm allocation). If at screening a recent chest CT scan has already been measured as part of the standard of care in the diagnosis of MABS, an additional chest CT will not be required. For Observational Cohort participants, a chest CT scan performed within six months of screening is acceptable. For Intervention Program participants, a chest CT scan must be performed within six months although ideally within a window of three months prior to screening to one week after intensive treatment has commenced. Once consent is provided for the FOR*Ma*T trial these images will be accessed from the participants medical records.

Chest CTs will be performed according to the study-specific SOPs, with steps taken to individualise scanning variables at each centre to ensure standardised lung volume (e.g. anaesthesia or breath hold), image quality and to minimize radiation exposure. Chest CTs can be used for clinical management, however, only site-generated reports, NOT the PRAGMA-CF score, will be available to clinicians.

The development and implementation of site-specific chest CT SOPs as well as the evaluation of trial CTs will be undertaken by the LungAnalysis team at Erasmus MC and are described in Appendix B3, Radiology.

To optimise volume levels during scanning in awake patients, training prior to the scanning by a technician is needed. The same technician instructs the patients during the scanning. When possible, the training will be executed with a spirometer and only in sites already trained in spirometry-controlled CT measurements and depending on the cooperation level of the participant. Instruction and demonstration of these procedure will be available on the FOR*Ma*T trial website along with the site-specific CT protocol in Appendix D.

5.5.3 Whole Genome Sequencing

All MABS positive isolates will be stored and DNA extracted. MABS isolates will be transported to the Child Health Research Centre, South Brisbane for DNA extraction. At the International sites, DNA extraction will be performed in accordance with the procedures outlined in Appendix D. Both Australian and International extracted DNA samples will be transported to the South Australian Health and Medical Research Institute (SAHMRI) for whole genome sequencing (WGS). Refer to Appendix D for WGS SOPs.

5.5.4 Spirometry

Spirometry will be measured in all participants from three years of age in accordance with the American Thoracic Society and European Respiratory Society standards (39). Spirometry will be measured at screening and at study visits at week 6, week 12, at the final study visit; either weeks 56 or 62 (depending on treatment arm allocation) and, if applicable at the early termination visit.

The multi-ethnic global lung initiative (GLI) references values for all spirometry indices will be applied to participants 3-95 years of age (40). Spirometry variables from the best reported flow volume loop will be recorded in the CRF.

5.5.5 Physical Examination

A physical examination of all body systems and vital signs will be completed in accordance with Table 1; at screening, at week 6, week 28 and at the final visit as well as with assessment of a severe adverse event. A physical examination is to be completed by the FOR*Ma*T trial physician. The physical examination includes a review of the following systems: head/neck/thyroid; eyes/ears/nose/throat; respiratory; cardiovascular; lymph nodes; abdomen; skin, musculoskeletal; and neurological systems. Breast, anorectal, and genital examinations will be performed only when medically indicated. After screening, any clinically significant abnormal findings in physical examinations will be reported as adverse events (AEs), see section 5.9.1.

Vital signs include blood pressure (systolic and diastolic), temperature (oral), pulse rate, and respiration rate will be assessed following a 5-minute rest in the seated or supine.

5.5.6 Medication Review

Current medication use including both prescription, over-the counter medication, herbal remedies and preparations will be recorded in accordance with the schedule outlined in table 1. Trade drug names, start and stop dates, dose, route and indications for use should be recorded in the CRF.

5.5.7 MABS-PD Status Review

Participants enrolled in the observational cohort only are required to have their MABS-PD status reviewed in accordance with the ATS criteria for MABS-PD diagnosis (section 4.3.1). The MABS-PD status of observational cohort participants is required to be reviewed at baseline, week 6, week 12, week

56 (final outcome) and, if applicable the early termination visit (see table 1) by the treating physician. This information is to be documented in the MABS-PD status CRF.

5.5.8 Health Related Quality of Life (HRQoL)

HRQoL will be assessed in all participants at baseline, week 6, week 12, week 18, week 28 and at the final outcome (week 56 or week 62 depending on randomisation to treatment arm). The questionnaires are required to be completed prior to any clinical assessment and are dependent on the age and the CF diagnosis (yes/no) of the participant as outlined below. The age appropriate HRQoL questionnaire issued to the participant at the start of the study are to continue being used throughout the study even if the participant progresses to a different age range. All HRQoL questionnaires can be found in Appendix D. All questionnaires will be made available in English and where possible translated into additional languages as required.

5.5.8.1 Cystic Fibrosis Questionnaire-Revised

The cystic fibrosis questionnaire-revised (CFQ-R) has been developed specifically for use in people with CF. This questionnaire measures the impact of CF on overall health, daily life, perceived well-being and symptoms. Age appropriate questionnaires have been developed; CFQ-R teen/adult for adolescents and adults 14 years of age and older, the CFQ-R child and the CFQ-R parent are to be completed by the child (6-13 years of age) and the parent/carer respectively of children with CF.

In children, ≤16 years of age the CFQ-R should be administered after the PedsQL™.

5.5.8.2 EQ-5D-5L

The EQ-5D-5L questionnaire is a standardised measure of health status in adults 18 years of age and older. This questionnaire can be applied as a generic measure of health for clinical and economic appraisal, including the calculation of quality-life adjusted years (QALY's). The EQ-5D-5L measures five dimensions:

1- Mobility;
2- Self-care;
3- Usual activities;
4- Pain/discomfort;
5- Anxiety/depression.

Each dimension has five possible answers; no problems, slight problems, moderate problems, severe problems and extreme problems. The respondent is asked to indicate his/her health state by selecting the most appropriate statement.

5.5.8.3 EQ-5D-Y

The EQ-5D-Y is the child friendly version of the EQ-5D-5L questionnaire. The EQ-5D-Y has been developed for use in children 8 to 17 years of age. The dimensions and the visual analogue scale (VAS) measured are the same as the EQ-5D-5L questionnaire, but with child friendly wording. For children 4-7 years of age the proxy 1 version of the EQ-5D-Y can be utilised allowing the respondent (parent/carer) to evaluate participants QOL from respondents' own view.

5.5.8.4 St. George Respiratory Questionnaire (SGRQ)

The SGRQ is to be completed by all non-CF participants 18 years of age and older. The SGRQ is a supervised self-administered 50-item questionnaire measuring health status across three domains; symptoms, activity and impacts (psycho-social) in people with airway obstruction.

5.5.8.5 Short Form- 36 Health Survey

The Short form-36 (SF-36) health survey is a self-reported questionnaire applicable in all participants 16 years and older. Covering eight health concepts the SF-36 health survey is a generic outcome measure designed to examine a person's perceived health status.

5.5.8.6 Pediatric Quality of Life Inventory (PedsQL™)

The Peds-QL™ child health questionnaire is a non-preference based measure to assess HRQoL for children and adolescents 2 to 16yrs of age. Developmentally appropriate child self-report questionnaires are available (ages 5-7, 8-12, 13-18) together with parent/carer proxy-reports (ages 2-4, 5-7, 8-12 and 13-18). If feasible, the PedsQL™ should be completed *before* the respondents complete any other health data forms and *before* they see their physician or healthcare provider.

Parents/carers, children (aged 8-12) and teenagers (13-18 years of age) may self-administer the PedsQL™ after the FOR*Ma*T site researcher has provided instructions. If it is determined by the FOR*Ma*T site researcher that the child, teen or parent/carer is unable to self-administer the PedsQL™ the questionnaire should be administered, word for word by the FOR*Ma*T site researcher. If the child has difficulty understanding the age appropriate PedsQL™ the preceding age questionnaire may be used. The parent and the child must complete the questionnaires independently of each other and in accordance with the PedsQL™ administration guidelines detailed in Appendix D.

5.5.8.7 Child Health Utility 9D

The Child Health Utility 9D (CHU9D) is a generic preference-based measure of paediatric HRQoL for use in children 7 to 17 years of age. The use of a descriptive system and a set of preference weights allows for the calculation of quality adjusted life years (QALYs) for use in economic evaluation.

5.6 Schedule of Assessments- Core Procedures

FOR*Ma*T trial participants, regardless of whether they are enrolled in the observation study or the intervention program are required to contribute data and sample collection as described below in Table 1. All study visits will be scheduled and coordinated by the FOR*Ma*T trial site researcher with all study visits to be completed on site. Acceptable study visit windows are outlined in Table 1 and can be calculated using the FOR*Ma*T visit date calculator spreadsheet. The calculator can be found on the FOR*Ma*T trial website and will be emailed to sites following the site initiation visit (SIV). Instructions on the use of the calendar are contained within the visit date calculator spreadsheet.

Additional Appendix specific procedures are described in the corresponding Appendix.

Table 1 FOR*Ma*T Core Trial Procedures

ASSESSMENT	SCREENING VISIT								FINAL OUTCOME				EARLY WITHDRAWAL VISIT
									6 Week Intensive		Prolonged Intensive		
	Day 0	**Week 4**	**Week 6**	**Week 10**	**Week 12**	**Week 18**	**Week 28**	**Week 38**	**Week 52 #**	**Week 56 #**	**Week 58 #**	**Week 62 #**	
Observation Cohort Participant Timeframes	-42 days	+14 Days	+14 Days	+14 Days	±14 Days	±14 Days	±14 Days	±14 Days	N/A	±14 Days	N/A	N/A	
Intervention Program Participant Timeframes	-42 days	±3 Days	±3 Days	±3 Days	±3 Days	±5 Days	±5 Days	±5 Days	+5 Days	+14 Days	+5 Days	+14 Days	
Clinic Visit[C]	✓	✓ñ	✓	✓ ñ	✓	✓	✓	✓	✓	✓	✓	✓	✓
Informed Consent	✓												
Review Eligibility	✓												
MABS-PD Status	✓		✓M		✓M					✓M			✓
Medication Review	✓		✓		✓	✓	✓	✓		✓		✓	✓
Respiratory Sample	✓	✓ x3^		✓ x3^		✓NP	✓NP	✓NP	✓NP	✓BAL	✓NP	✓BAL	✓
Height and Weight[†]	✓	✓ɸ	✓	✓ɸ	✓	✓	✓	✓	✓	✓	✓	✓	✓
Spirometry	✓		✓		✓					✓		✓	✓

Chest Computed Tomography	✓				✓[&] Optional					✓		✓	✓[$]
Physical Examination	✓		✓				✓			✓		✓	✓
EQ-5D-5L [E]	✓		✓		✓	✓	✓			✓		✓	✓
SF-36 [G]	✓		✓		✓	✓	✓			✓		✓	✓
PedsQL ™[##]	✓		✓		✓	✓	✓			✓		✓	✓
CFQ-R *	✓		✓		✓	✓	✓			✓		✓	✓
EQ-5D-Y [Q]	✓		✓		✓	✓	✓			✓		✓	✓
Child Health Utility 9D [U]	✓		✓		✓	✓	✓			✓		✓	✓
SGRQ [S]	✓		✓		✓	✓	✓			✓		✓	✓

[#] Intervention program participants are required to complete assessments until either week 52 and 56 (participants randomised to 6 weeks of intensive therapy) OR week 58 and 62 (those participants randomised to prolonged intensive therapy), not both. The final outcome for the observational cohort will be assessed at week 56.

[C] Additional clinic visits are required for FOR*Ma*T participants enrolled in the Intervention Program.

[fi] Clinic visit at Week 4 and week 10 not required for participants enrolled in the Observational Cohort group.

[M] Only participants enrolled in the observational cohort are required to have their MABS-PD status reviewed in accordance with the ATS criteria.

^ Intervention Program participants are required to provide three sputum samples or one BAL sample for microbiology assessment as described above. during both week 4 and week 10 (±3 days) as described in section 5.5.1. Observational Cohort participants are requested to provide three sputum samples between week 4 to week 6 as well as between week 10 to week 12, and if they are unable to provide a sputum sample, one BAL is to be collected at Week 10 for microbiology assessment.

[NP] Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.

[BAL] Participants are requested to provide monthly sputum samples from Week 14. If the participant is unable to provide monthly sputum samples, then participants are requested to provide a minimum of four sputum samples(timepoints suggested in table). If participants are unable to provide the minimum

requested sputum samples detailed in the table, then a BAL sample is to be collected during Week 56 (for observational participants and short intensive participants) or Week 62 (for prolonged intensive participants).

[†] Adult participants require height to be recorded once only during the study (ideally at the Screening Visit).

[ɸ] Adult participants do not require weight to be measured at Week 4 or Week 10. Paediatric participants require height and weight to be measured at Week 4 and Week 10.

[&] Week 12 CT Scan is optional and forms part of FOR*Ma*T Sub-Study B3: Imaging. The site must have approval to conduct this additional scan and the participant must provide additional consent.

[$] Chest CT Scan at early withdrawal visit will only be requested if clinically indicated.

[E] The EQ-5D-5L is to be assessed in participants ≥18 years of age

[G] The SF-36 is to be assessed in participants ≥16 years of age.

[# #] The PedsQL is only to be assessed in participants ≤16 years of age. If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R. The age appropriate questionnaire should be selected.

***** The CFQ-R is only to be completed in participants with CF. The age appropriate CFQ-R assessment should be selected; CFQ-R adult/teen, CFQ-R child and CFQ-R parent.

[Q] The EQ-5D-Y is to be assessed in participants 8-17 years of age.

[U] The Child Health Utility 9D is to be assessed in participants 7 to 17 years of age.

[S] The SGRQ is to be assessed in non-CF participants ≥18 years of age.

5.7 Quality Assurance (QA)

The FOR*Ma*T trial will be conducted in accordance with the current approved Master Protocol. To improve Master Protocol and SOP adherence as well as complete data entry the following QA procedures will be implemented:

- A FOR*Ma*T trial start-up meeting for research coordinators and site investigators prior to the submission of ethical approval and governance documents.
- A SIV once the FOR*Ma*T site has obtained ethics and governance approval, but prior to recruitment as described above.
- A CRF Completion Guide detailing all the data to be collected in the CRFs/eCRFs.
- Regular and timely validation of data entered, queries and corrections by the FOR*Ma*T Trial Management Team.
- Trial monitoring (onsite, remote, central, and/or local) as described in Section 7.6 Site Monitoring.

5.7.1 Microbiology and DNA Extraction QA

Microbiology is of key importance and samples are processed in each of the participating countries in the national mycobacterial reference laboratories (MRL). Prior to international sites in any one country commencing, the participating MRLs for the country will be required to have:

1- Accreditation from the appropriate national body;
2- Evidence of meeting appropriate International Organization for Standardization (ISO) 15189 Standards;
3- Evidence of participation in an external proficiency testing program which includes MABS;
4- Evidence of satisfactory performance in Quality Assurance Program (QAP) which would include raw results and evidence of corrective actions for discordance – perfect scores not required but quality improvement process is;
5- Review of SOPs with respect to MABS and NTM isolation and drug susceptibility testing (DST) from respiratory samples.

A review panel chaired by Dr Christopher Coulter (PI) Director of the Queensland Mycobacterial Reference Laboratory and World Health Organization Collaborating Centre for Tuberculosis and SupraNational Reference Laboratory will ensure standard approaches are followed. The FOR*Ma*T Trial Management Team will also work with each MRL to ensure understanding of the SOPs and requirements for sample storage and DNA extraction.

To ensure consistency in DNA extract preparation, test samples based on cultured type strains will be submitted for quality assessment by the team at SAHMRI, Australia, led by Professor Geraint Rogers, where the trial analysis of WGS will be performed.

5.8 Notes on Specific Trial Visits

5.8.1 Screening (Day 0, visit window*: -42 days to Day 0)

During the screening visit the participants eligibility into the trial will be assessed in accordance with the eligibility outlined in section 4.1. If, during the screening visit the participant is eligible for the intervention program and consent is obtained, additional screening assessments described in Appendix A are required to be completed. *Exceptions to the screening visit window include respiratory samples collected within six months for all FOR*Ma*T Trial participants and ideally within three months for Intervention Program participants and chest CT scans measured within six months prior to the screening visit.

5.8.2 Final Outcome Study Visit

For observational cohort participants, the final outcome visit will take place at week 56. For participants enrolled in the Intervention program the final outcome study visit will take place four weeks after consolidation therapy is ended; at either week 56 or week 62 (depending on the treatment arm allocation). Serious adverse events (SAEs) that are still present at the final study visit should, where possible, be followed by the site Investigator in accordance with section 5.9.4 until a final assessment can be made.

5.8.3 Early Withdrawal Visit

Participants who are withdrawn or who withdraw from the trial will be asked to attend an Early Withdrawal Visit. Where practical, all efforts should be made for the participants to complete the early withdrawal trial procedures detailed in Table 1 Schedule of Assessments within one week of withdrawing. If there are any ongoing adverse events (AEs) these will be followed and monitoring in accordance with the FOR*Ma*T Master Protocol irrespective of withdrawal from study.

5.8.4 Unscheduled Visit

Study visits that are conducted in addition to those listed in Table 1 and are known as Unscheduled Visits. If the Investigator deems that a participant should attend the study site to follow up an AE, repeat laboratory testing or other reason, then this visit should be documented in Unscheduled Visit case report form (CRF).

5.9 Safety Monitoring

Occurrence of AEs due to the underlying MABS infection and treatments used are well recognised and expected during the FOR*Ma*T trial. As such, toxicology monitoring procedures outlined in Appendix A will be implemented for participants enrolled in the intervention program.

5.9.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An

AE is therefore any unfavourable and unintended sign (including any clinically significant abnormal laboratory finding/s), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the participant's source documents. AEs will also be recorded in the participant CRF. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study medication, or if unrelated, the cause.

AEs will be collected from the time of informed consent until 30 days after the final study visit.

5.9.2 Attribution of Adverse Events

The relationship, or attribution, of an AE to the trial therapies will be determined by the Investigator. The relationship of the AE to the investigational product should be coded according to the following definitions:

Unrelated: The adverse event is clearly not related to the investigational product.

Unlikely: The adverse event is doubtfully related to the investigational product.

Possibly: The adverse event may be related to the investigational product.

Probably: The adverse event is likely related to the investigational product.

Definitely: The adverse event is clearly related to the investigational product.

5.9.3 Definition of Serious Adverse Events

SAEs will be defined as any untoward medical occurrence that at any dose:

- Results in death.
- Is considered life threatening (i.e., in the view of the Investigator the adverse experience places the participant at immediate risk of death from the reaction, as it occurred; it **does not** include a reaction that, had it occurred in a more severe form, might have caused death).
- Requires hospital admission or prolongation of an existing hospitalisation.
- Results in persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions).
- Is a congenital anomaly/birth defect.
- Is an important medical event (i.e., when based upon appropriate medical judgment, the adverse experience may jeopardise the participant and may require medical or surgical intervention to prevent one of the above listed outcomes).

SAEs that are still present at the end of the study should be followed up by the site Investigator until a final assessment is possible by the treating physician.

5.9.4 Reporting of Safety Events

Study sites will document all SAEs that occur after informed consent is obtained until 30 days after the final study visit in an SAE Report (whether or not related to study treatment). All SAEs will be reviewed by the site Investigator and reported to the FOR*Ma*T Trial Management Team within 24 hours of learning of the event. Exclusions to the SAEs expedited reporting requirements of the FOR*Ma*T Trial include: a) infective exacerbation of an underlying lung condition requiring hospitalisation or home intravenous antibiotic treatment, b) any planned and/or elective hospital admissions or c) any planned and/or elective medical procedures.

The site will complete and submit an initial SAE report form via email or the FOR*Ma*T trial database. Any follow up information about the SAE is to be reported on an SAE report form and sent via email or submitted in the FOR*Ma*T trial database as soon as relevant information is available.

All SAEs will be reviewed by the FOR*Ma*T trial Pharmacovigilance Team and coded in accordance with the CTCAE version 5. For any SAE that is unable to be categorised in accordance with the CTCAE, the independent data safety monitoring board (iDSMB) will be required to review and categorise the SAE.

Suspected unexpected serious adverse reactions (SUSAR's) will be reported on the SAE form and emailed to the FOR*Ma*T Trial Management Team or submitted via the FOR*Ma*T Trial database. Urgent safety measures (USMs) and significant safety issues (SSIs) will be reported on the Significant Safety Issue report form and emailed to the FOR*Ma*T Trial Management Team or submitted in the FOR*Ma*T trial database. USMs, SSIs and SUSARs will be reported by the FOR*Ma*T Trial Management Team to the relevant IRB/IEC/HREC.

The FOR*Ma*T Trial Management Team will notify the Sponsor of all SUSARs, USMs, SSIs and submit an Annual Safety Report. The FOR*Ma*T Trial Management Team and/or delegates will notify the relevant IRB/IEC/HREC of any USMs or SSIs and submit an Annual Safety Report.

Site investigators are required to report all safety critical AE's (Table 2) and laboratory evaluations to the FOR*Ma*T Trial Management Team within 24 hours for abnormalities corresponding to CTCAE Grade 3 or higher and within two weeks for abnormalities corresponding to CTCAE Grade 1 or 2.

Detailed procedures for safety monitoring and reporting are outlined in FOR*Ma*T SOP 6.

5.9.5 Coding of Adverse Events for Analysis

CTCAE, version 5.0 (Appendix D) will be applied for the database coding of AEs including SAEs categorised as at least "possibly-", "probably-", or "definitely-" related to study medications and/or interactions between study medications and concomitant medications. Coding for analysis will be completed by the FOR*Ma*T Pharmacovigilance Team. "Refer to section 4.5 for the definition of tolerance.

If the severity of an AE is unable to be coded in accordance with the CTCAE the iDSMB will be consulted to assess and grade the AE.

5.9.6 Toxicology Thresholds

Toxicology monitoring will use the following thresholds that match the grading from CTCAE, version 5.0 as described in table 2. Toxicity monitoring is being done in the local sites for intervention programs using guideline-based therapies and results will be entered by the local site team into the trial database. If abnormalities are noted that correspond with a Grade 1 or 2 level and are deemed to be clinically significant, the investigators will be required to respond to a toxicity query that asks for an AE report to be completed within two weeks. If abnormalities correspond with a Grade 3 or higher level, an urgent critical safety alert will be sent to the investigator requiring an adverse event report to be completed within 24 hours.

The AE report will also include plans for interruption or cessation of study treatments and ongoing monitoring. The decision to interrupt treatment will be made by the local investigator.

New interventions in sponsored trials will require a separate toxicity monitoring plan including any requirements for central laboratory testing of samples.

5.10 Participant Withdrawal and Discontinuation of Treatment

5.10.1 Withdrawal of Consent

All participants are free to withdraw from the study at any time point, with or without a specified reason and without prejudice. Where possible, the participant or the participant's parent/legal guardian is required to sign the withdrawal of consent form, formally documenting the withdrawal process including the reason for withdrawal if they choose to provide this information. In the event of a participant withdrawing from the trial an early termination visit is to be completed within one week of withdrawing. If, for any reason the participant is unable to complete the early termination visit this should be noted on the CRF and recorded in the AE.

5.10.2 Discontinuation of Treatment

Participants enrolled in the intervention program can discontinue treatment at any time during the trial if:

- The participant is no longer able to comply with the FOR*Ma*T Master Protocol, including completing required study assessments for safety requirements.
- The Investigator believes that treatment is no longer in the participants' best interests (due to safety or tolerance concerns).
- The participant no longer wants to continue treatment.

Participants discontinuing treatment will be encouraged to continue with the schedule of assessments in accordance with the Master Protocol.

Table 2: Safety critical monitoring thresholds

Investigations					
	Grade				
CTCAE Term	**1**	**2**	**3**	**4**	**5**
Alanine aminotransferase increased	>ULN - 3.0 x ULN if baseline was normal, ***or;*** 1.5 – 3.0 x baseline if baseline was abnormal.	>3.0 - 5.0 x ULN if baseline was normal, ***or;*** >3.0 - 5.0 x baseline if baseline was abnormal.	>5.0 - 20.0 x ULN if baseline was normal, ***or;*** >5.0 - 20.0 x baseline if baseline was abnormal.	>20.0 x ULN if baseline was normal, ***or;*** >20.0 x baseline if baseline was abnormal.	-
Definition: A finding based on laboratory test results that indicate an increase in the level of alanine aminotransferase (ALT or SGPT) in the blood specimen.					
Anaemia	Haemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN – 6.2 mmol/L; <LLN – 100 g/L.	Hgb <10.0 – 8.0 g/ dL; <6.2 – 4.9 mmol/L; <100 - 80 g/L.	Hgb <8.0 g/ dL; <4.9 mmol/L; < 80 g/L; transfusion indicated.	Life-threatening consequences; urgent intervention indicated.	Death
Definition: A disorder characterized by a reduction in the amount of haemoglobin in 100 ml of blood. Signs and symptoms of anaemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart, soft systolic murmurs, lethargy, and fatigability.					
Aspartate aminotransferase increased	>ULN - 3.0 x ULN if baseline was normal, ***or;*** 1.5 – 3.0 x baseline if baseline was abnormal.	>3.0 - 5.0 x ULN if baseline was normal, ***or;*** >3.0 – 5.0 x baseline if baseline was abnormal.	>5.0 - 20.0 x ULN if baseline was normal, ***or;*** >5.0 – 20.0 x baseline if baseline was abnormal.	>20.0 x ULN if baseline was normal, ***or;*** >20.0 x baseline if baseline was abnormal.	-
Definition: A finding based on laboratory test results that indicate an increase in the level of aspartate aminotransferase (AST or SGOT) in the blood specimen.					
Blood bilirubin increased	>ULN - 1.5 x ULN if baseline was normal, ***or;*** >1.0 – 1.5 x baseline if baseline was abnormal.	>1.5 - 3.0 x ULN if baseline was normal, ***or;*** >1.5 – 3.0 x baseline if baseline was abnormal.	>3.0 – 10.0 x ULN if baseline was normal, ***or;*** >3.0 – 10.0 x baseline if baseline was abnormal.	>10.0 x ULN if baseline was normal, ***or;*** >10.0 x baseline if baseline was abnormal.	-
Definition: A finding based on laboratory test results that indicate an abnormally high level of bilirubin in the blood. Excess bilirubin is associated with jaundice.					
Electrocardiogram QT corrected interval prolonged	Average QTc 450 - 480 ms	Average QTc 481 - 500 ms	Average QTc >= 501 ms; >60 ms change from baseline.	Torsade de pointes; polymorphic ventricular tachycardia; signs/symptoms of serious arrhythmia.	-
Definition: A disorder characterised by Electrocardiogram T wave amplitude changes.					

Investigations					
	Grade				
CTCAE Term	**1**	**2**	**3**	**4**	**5**
Hearing impaired	**Adults** enrolled on a Monitoring Program (on a 1, 2, 4, 3, 6, and 8 kHz audiogram): Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear; **Adults** not enrolled on a Monitoring Program: Subjective change in hearing in the absence of documented hearing loss; **Pediatric** (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift >20 dB hearing loss (HL) (i.e., 25 dB HL or greater); sensorineural hearing loss (SNHL) above 4 kHz (i.e., 6 or 8 kHz) in at least one ear.	**Adults** enrolled on a Monitoring Program (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least one ear; **Adults** not enrolled on a Monitoring Program: Hearing loss with hearing aid or intervention not indicated; limiting instrumental ADL; **Pediatric** (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift >20 dB at 4 kHz in at least one ear.	**Adults** enrolled on a Monitoring Program (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear; therapeutic intervention indicated; **Adults** not enrolled on a Monitoring Program: Hearing loss with hearing aid or intervention indicated; limiting self care ADL; **Pediatric** (on a 1, 2, 3, 4, 6, and 8 kHz audiogram): Hearing loss sufficient to indicate therapeutic intervention, including hearing aids; threshold shift >20 dB at 2 to < 4 kHz in at least one ear.	**Adults**: Decrease in hearing to profound bilateral loss (absolute threshold >80 dB HL at 2 kHz and above); nonservicable hearing. **Pediatric**: Audiologic indication for cochlear implant; > 40 dB HL (i.e., 45 dB HL or more); SNHL at 2 kHz and above.	-
Definition: A disorder characterized by partial or complete loss of the ability to detect or understand sounds resulting from damage to ear structures					
Leukocytosis	-	-	>100,000/mm^3	Clinical manifestations of leucostasis; urgent intervention indicated.	-
Definition: A disorder characterised by laboratory results that indicate an increased number of white blood cells in the blood.					

Investigations					
	Grade				
CTCAE Term	**1**	**2**	**3**	**4**	**5**
Lymphocyte count decreased	<LLN - 800/mm^3; <LLN - 0.8 x 10^9/L	<800 – 500/mm^3; <0.8 - 0.5 x 10^9/L	<500 – 200/mm^3; <0.5 - 0.2 x 10^9/L	< 200/mm^3; < 0.2 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the number of lymphocytes in a blood specimen.					
Neutrophil count decreased	<LLN - 1500/mm^3; <LLN - 1.5 x 10^9/L	<1500 - 1000/mm^3; <1.5 - 1.0 x 10^9/L	<1000 - 500/mm^3; <1.0 - 0.5 x 10^9/L	<500/mm^3; < 0.5 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the number of neutrophils in a blood specimen.					
Platelet count decreased	<LLN - 75,000/ mm^3; <LLN - 75.0 x 10^9/L	<75,000 - 50,000/ mm^3; <75.0 - 50.0 x 10^9/L	<50,000 - 25,000/ mm^3; <50.0 - 25.0 x 10^9/L	<25,000/ mm^3; <25.0 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the levels of pancreatic enzymes in a biological specimen.					
Tinnitus	Mild symptoms; intervention not indicated.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self care ADL.	-	-
Definition: A disorder characterised by noise in the ears, such as ringing, buzzing, roaring or clicking.					
Vertigo	Mild symptoms.	Moderate symptoms; limited instrumental ADL.	Severe symptoms; limiting self care ADL.	-	-
Definition: A disorder characterised by a sensation as if the external world were revolving around the patient (objective vertigo) or as if he himself were revolving in space (subjective vertigo).					
Vestibular Disorder	-	Symptomatic; limiting instrumental ADL.	Severe symptoms; limiting self care ADL.	-	-
Definition: A disorder characterised by dizziness, imbalance, nausea and vision problems.					
White blood cell decreased	<LLN - 3000/ mm^3; <LLN – 3.0 x 10^9/L	<3000 - 2000/ mm^3; <3.0 – 2.0 x 10^9/L	<2000 - 1000/ mm^3; <2.0 – 1.0 x 10^9/L	<1000/ mm^3; <1.0 x 10^9/L	-
Definition: A finding based on laboratory test results that indicate a decrease in the number of white blood cells in a blood specimen.					

Adapted from CTCAE, version 5.0

5.11 Data Management

5.11.1 Data Collection, Entry and Storage

Trial data will be collected from various sources including, but not limited to medical records, participant questionnaires, output from lab results, study assessments, correspondence and CT scans. These documents and any documents where data is first recorded for a participant will form the source documents. FOR*Ma*T trial site staff will be trained in the collection of the required data during the site initiation visit and data collection instructions will be stored in the site master file and on the trial website.

The site Investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of all trial data reported. All source documents are to be completed in a neat, legible manner to ensure accurate interpretation of data. The site Investigators will maintain adequate case histories of trial participants, including source documentation. The Investigators will maintain a record that details the location of essential documents, including those documents stored outside the Investigator Site File.

Sites will have the option to enter the data required for the trial into either a paper based CRF or, where possible into an electronic CRF (eCRF). Data for the CRF or eCRF will be obtained directly from the source documents. For sites using the paper CRF, data will then be entered onto a study specific REDCap FOR*Ma*T database, which will be accessible by the FOR*Ma*T trial team only. Alternatively, if using the eCRF data can be entered and uploaded directly into the REDCap FOR*Ma*T database.

It is crucial to the adaptive design of the trial that **data is entered as soon as possible after the study visit**. Data cleaning will be performed weekly for the trial to ensure that the data are cleaned and ready for interim analyses.

The REDCap FOR*Ma*T database will be hosted on Murdoch Children's Research Institute (MCRI) infrastructure and is subject to the same security and backup regimen as other systems at the MCRI (e.g. the network file servers). Data is backed up nightly to a local backup server, with a monthly backup taken to tape and stored offsite. All data transmissions between users and the REDCap server are encrypted. Regular data quality checks, such as automatic range checks, will be performed to identify data that appear inconsistent, incomplete, or inaccurate.

Access to REDCap is via an MCRI user account or (for external collaborators) via a REDCap user account created by the system administrator. The permissions granted to each user within each REDCap project is controlled by and is the responsibility of the project team. REDCap has functionality that makes adding and removing users and managing user permissions straightforward. REDCap maintains an audit trail of data create/update/delete events that is accessible to project users that are granted permission to view it.

5.11.2 Data Storage and Retention

Each site is required to maintain source documents for a minimum of 15 years post completion of the trial. If site specific requirements dictate data retention for periods longer than 15 years, trial sites are required to adhere to these requirements.

6 STATISTICAL ANALYSIS PRINCIPLES

This section of the Master Protocol provides an outline and summary of the general statistical methods and principles used for FOR*Ma*T. The detailed statistical methods and sample size simulations relevant to each of the intervention programs and to discovery studies are outlined in the relevant Appendices.

6.1 Bayesian Analysis and Bayesian Adaptive Randomisation (BAR)

Interim monitoring for the intervention program in the FOR*Ma*T trial will use a Bayesian analysis approach (41). Under this approach we will calculate the (posterior) probability of an intervention being found to be superior to the reference arm during the trial. As new data is generated the probability will be updated. This updating will occur at the time of the iDSMB meetings who will have access to unblinded data and will be asked to ratify the updated probabilities. Initially there are no plans to stop any arms of the study arms due to superiority, but such rules may be established as the trial continues and will be detailed in the relevant appendix. The iDSMB may however make a recommendation about stopping current interventions if they show poor promise or futility. Stopping rules will be defined to guide the use of these posterior probabilities.

Adaptive randomisation allows intervention arm allocation ratios to be adapted based on interim analyses undertaken during the trial to favour the intervention arm with the highest posterior probability of success (42). This approach can lead to increased efficiency of the trial and reduce patient exposures to less promising or more toxic therapies. In the FOR*Ma*T trial, BAR will be used for updating allocation probabilities in randomisations when there are more than two interventions being compared at any one of the randomisation stages. BAR will be implemented after every 100 participants have been randomised so that there is sufficient information available to determine the adaptation. Success of an intervention (and hence the adaptations) will be determined by the primary outcome, microbiological clearance with tolerability.

For the first intervention program (Appendix A1), BAR will initially just be used in the intensive phase, but as treatments are added into different stages it may also be used at other randomisation stages. In the intensive phase, after achieving the minimum number of 100 subjects to be recruited into this randomisation stage, BAR will be implemented at two interim updates (after 100 and 200 participants have been randomised), unless the data overwhelmingly support early stopping for futility, or the maximum sample size is attained (see simulation (sample size) Appendix A1, section 12).

6.2 Randomisation

6.2.1 Blinding of Treatment Allocation

The FOR*Ma*T trial may include placebo controlled double blind randomised interventions in the future, but the initial intervention program is randomised but open label.

There will be three stages of randomisation in the intervention program of this study, dictating the treatment the participant will receive:

***Randomisation 1*:** The first randomisation will be at the start of the intensive phase, with all participants randomised between the different intensive therapy arms for a period of 6 weeks.

Randomisation 2: The second randomisation will ONLY be for participants who are still MABS positive at week six and are able to continue with intensive therapy. Randomisation will occur at the end of week 6 and will allocate participants to either;

1. Continue intensive therapy which will be followed by consolidation, or;
2. Immediately commence consolidation therapy.

Randomisation 3: This randomisation will allocate participants to the consolidation therapy arms either at week 6 or at week 12 depending on the most recent MABS clearance result, i.e. from week six or (if applicable) from week 12 following prolonged intensive therapy.

Week 6: This randomisation occurs at 6 weeks for patients who have cleared MABS at 6 weeks and for patients who remained MABS positive at 6 weeks and were randomised in randomisation 2 to start consolidation. Participants who remain MABS positive after 6 weeks of treatment but who are unable to continue with any IV treatment due to serious adverse event may proceed directly to Randomisation 3.

Week 12: Patients who are randomised to prolonged intensive therapy will be randomised between the consolidation arms at the end of week 12.

Each randomisation will function as a 'quasi-separate' trial (as described in Appendix A1), as well as being considered in combination (intensive + consolidation). Randomisation at each level will be conducted using the method of minimisation (described below). Each randomisation level will be planned to enable flexibility via pre-planned adaptations as described above.

6.3 Minimisation

To ensure balance between arms in important patient characteristics, randomisation at the 3 different stages outlined above will use minimisation with a random element. Minimisation is a dynamic randomisation approach used in clinical trials to balance allocation to treatment arms with respect to a number of important stratification factors. In minimisation, the first participant is allocated to their

treatment arm at random. Subsequent participants are assigned to a treatment arm by first selecting the preferred arm that would best improve the balance of participants across the arms based on the stratification variables of interest in terms of the numerical difference in the sample size in each of the treatment arms across all of the stratification factors (43, 44). The preferred arm is then selected with a probability of 0.7, with rest of the probability split between the alternative arms.

Following updating the randomisation allocations through BAR, minimisation will be conducted using Biased Coin Minimisation (BCM) (45). Under this method, the same methodology as standard minimisation will be used to determine the preferred intervention, but the randomisation probabilities will be altered to reflect the minimisation probabilities and the updated allocation ratios from the BAR as detailed in Han et al (45).

Randomisation will be conducted electronically through the trial database following completion of all the specific required data entry by the study team at each site. Participants will be randomised according to the stratification criteria described below using to the weights specified for each factor:

1. Macrolide resistance*: Yes or no (weight = 50% in randomisations 1 and 2, 25% in randomisation 3).
   Any of these measurement methods are acceptable for defining macrolide resistance (in order of preference):
   a. Inducible at 14 days or constitutive at 3 days;
   b. *Erm*(41) status: Functional or dysfunctional;
   c. MABS subspecies: *M. a. abscessus* & *M. a. bolletii* combined or *M. a. massiliense*.
2. Age: <12 years, 12-30 years and >30 years of age (weight = 20%).
3. Sex: Male or Female (weight = 7.5%).
4. Location: Australia and New Zealand as one stratum, United Kingdom and Ireland as one stratum, Denmark, France and the Netherlands as another stratum and Canada as one stratum (weight = 7.5%).
5. Cystic Fibrosis Status: Yes or no (weight = 7.5%).
6. Mixed NTM infections at enrolment: Yes or no (weight =7.5%).
7. MABS positive culture (at initial randomisation to intensive therapy and for randomisation 2 all participants will have a positive culture, so this factor will not be required. However, it will be required for randomisation 3): Yes or no (weight = 25%).

6.4 Adding and Stopping Interventions

New interventions, either during the intensive or consolidation phase, may be added as a treatment arm either for the intensive or for the consolidation phase as determined by the trial drug selection committee

(DSC) with approval of the Trial Steering Committee (TSC). No new therapies will be added until at least 100 subjects have completed the trial phase associated with the proposed new intervention.

Interventions may be stopped early due to a lack of benefit at interim analyses. Interim analyses will be conducted after each 100 patients have completed the trial. The statistical team, acting in confidence will present the results from such analyses only to the iDSMB who will provide guidance around stopping particular interventions. Pre-defined triggers for stopping an intervention will be specified for both intensive and consolidation interventions separately, and for the combination of intensive and consolidation. If a single intervention, or combination of interventions, has less than a 0.01 posterior probability of being an optimal intervention in that phase then that intervention will be regarded as inferior and should be recommended to be discontinued.

For new interventions in the future, if an intervention has a high posterior probability (to be pre-specified prior to the new intervention being added) of being an optimal therapy this invention will be considered as superior, in which case the iDSMB might recommend that the randomization stage be stopped for superiority. Stopping rules for superiority will be detailed in the relevant section of the Appendix. Following the iDSMB meeting, the recommendations from the iDSMB will be notified to the trial monitoring committee (TMC) and TSC. The TSC will consider the recommendations and make a decision regarding the potential to stop the randomization stage for superiority. If any interventions are stopped, the TMC will have the responsibility of developing a public disclosure as is practical through presentation of results and publications.

6.5 Seamless Phase II to Phase III

The FOR*Ma*T standing platform trial provides a resource to enable promising therapies to be more rapidly evaluated and has the potential to facilitate seamless transition from Phase II to a Phase III study. Initially the platform will support a phase II study, but new interventions being evaluated in the future may be considered for the potential to move seamlessly from this Phase II study to a Phase III (46). The principles guiding this move will include planning for the potential of Phase III in the design of Phase II study and with agreement between the Platform trial team and sponsors of the new therapy around management and access to data. The move to a Phase III trial will be guided by a pre-defined threshold for the probability of a successful intervention during phase III, which would need to be established in planning the Phase II trial and agreed by regulatory authorities for pivotal studies. If a seamless transition to Phase III was considered feasible, the TMC along with the trial statistics team will provide the iDSMB and TSC with a Phase III proposal, and if this was approved by the iDSMB and TSC then further recruitment to the target number determined would be undertaken.

6.6 Simulations and Statistical Power

The design (including stopping rules**)** and sample size of the initial intervention program, as well as the design and sample size following the addition of any new interventions, is informed by **s**imulations

using Monte Carlo methods to give a range of power according to different scenarios, and taking into account the probabilities of variable responses to intensive and consolidation interventions. Simulations will be updated with the addition of any new interventions. The details of these simulations can be found in the relevant sections in Appendix A.

6.7 General Analysis Principles

6.7.1 Intention to Treat Analysis

All analyses will be conducted following the intention-to-treat principle. It is possible that participants in the intervention trial may cease one or more of the intervention treatments for their allocated arm because of toxicity and may need alternate therapy introduced. In addition, we recognise that clinicians will have concerns if patients remain culture positive through the duration of the trial and may wish to change or add treatments. All changes in the intervention treatment allocated will be captured through the study database and any adverse events will be reported. Irrespective of whether treatments are ceased or altered, participants will remain in their assigned arm for analysis. They may also continue into the next stage of the trial, for example a participant who was unable to tolerate intravenous tigecycline in the first intervention program (Appendix A1) might cease tigecycline and an alternative treatment may be included in the intensive therapy stage. However, once the intensive stage has been completed they might still be randomised at the randomisation stage 2 or 3. Change of treatment required for toxicity/AE or failure to clear MABS would be reflected in the primary outcome of clearance of MABS with good tolerance as they would fail to achieve this outcome for that stage of the trial.

6.7.2 Analysis Methods

Following the completion of any part of the trial, analyses will be conducted using standard frequentist methodology, with results reported as group differences along with 95% confidence intervals and p-values. For phase II components we will also conduct a Bayesian analysis that reports the predictive probability of a phase III trial being successful. Providing it is indicated, missing data will be handled using multiple imputation for all analyses. The exact statistical methods to be used are outlined in the relevant sections of the appendices. Analyses will be adjusted for the study strata used in randomisation.

6.7.3 Managing Carry-Over Effects

Simulations considering different scenarios for the probability of clearance with tolerability at the 3 randomisations takes into account the effects of different treatment pathways through the trial and thus address the issue of carryover effects on assessment of power in the trial. Carryover should also not affect the statistical properties of subsequent stages of treatment as we are randomising at each stage independently of the other stages. If it appears that treatments given at previous treatment stages affect

results of subsequent treatment stages, we will incorporate a statistical method that can adjust for this as a sensitivity analysis.

6.8 Managing Population Time Trends

BAR can be subject to time trends if the patient characteristics change throughout the trial. Recent work (47) has, however, shown that the change in treatment outcomes must be extremely high to affect the statistical properties more than a negligible amount. The analysis will, however, adjust for time to mitigate the effect of any potential trial trends.

7 STUDY OVERSIGHT

7.1 Overview

The FOR*Ma*T Trial will have several committees established to provide oversight of the FOR*Ma*T Trial. -

7.2 Trial Steering Committee

The TSC is the executive decision-making group and provides overall supervision of the FOR*Ma*T trial. The Terms of Reference (see Appendix D) outlines the roles and responsibilities of the TSC members. Membership to the TSC will consist of independent and non-independent members from a variety of backgrounds including clinical and statistical as well as a member from the general community. The Chair of the TSC will be independent of the FOR*Ma*T Trial.

7.3 Independent Data Safety Monitoring Board

The iDSMB will review interim analyses, monitor for effectiveness and safety along with trial conduct. They will meet prior to the commencement of the trial at least annually and at the time of interim analysis after every 100 participants have completed each stage of the trial. The iDSMB will provide advice to the TSC and the Trial Management Committee (TMC). A Charter (see Appendix D) that outlines the terms of reference with roles and responsibilities will be established prior to the first meeting of the iDSMB. Membership to the iDSMB will consist of clinicians and statisticians who are independent of the FOR*Ma*T Trial.

The iDSMB will be responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review and voting procedures prior to initiating any data review. The iDSMB will be responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it.

The iDSMB will review each version of the FOR*Ma*T Master Protocol for any major concern prior to implementation. During the trial, the iDSMB will review cumulative study data to evaluate safety, study

conduct, and scientific validity and integrity of the trial. As part of this responsibility, iDSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The iDSMB will also assess the performance of overall study operations and any other relevant issues, as necessary. Items to be reviewed by the iDSMB will include:

- Interim/cumulative data for evidence of study-related AEs;
- Interim/cumulative data for evidence of efficacy and futility according to pre-established statistical guidelines;
- Data quality, completeness, and timeliness;
- Performance of individual centres/countries;
- Adequacy of compliance with goals for recruitment and retention, including those related to the participation of women and minorities;
- Adherence to the protocol;
- Factors that might affect the study outcome or compromise the confidentiality of the trial data (such as protocol deviations, unmasking, etc.);
- Factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study.

The iDSMB will conclude each review with their recommendations to the TSC and the FOR*Ma*T PI as to whether the study should continue without change, be modified, or terminated. Recommendations regarding modification of the design and conduct of the study could include:

- Modifications of the Master Protocol based upon the review of the safety data;
- Suspension or early termination of the study or of one or more study arms because of serious concerns about subjects' safety, inadequate performance or rate of enrolment;
- Suspension or early termination of the study or of one or more study arms because study objectives have been obtained according to pre-established statistical guidelines;
- Corrective actions regarding a study centre whose performance appears unsatisfactory or suspicious.

Confidentiality must always be maintained during all phases of iDSMB review and deliberations.

Meetings will include an open session and a closed session. The lead investigators of FOR*M*aT can attend the open session along with other investigators as requested.

The closed session will only include the iDSMB members who have voting rights and they will formulate recommendations regarding the study to the TSC.

Reports will be provided to iDSMB that include an open report and a closed report. Reports for the iDSMB will be prepared by the data team at the MCRI.

Open reports will include information on the study conduct such as accrual data, demographics and baseline characteristics, site performance and protocol compliance and quality control issues along with general toxicity and safety data presented across the study as a whole with no reference to treatment arm.

Closed reports will include the same data as the open reports as well as data on efficacy outcomes at the time of each interim analysis (every 100 patients in randomisation levels with more than two interventions) and will be presented by (masked) treatment arm.

Reports from the iDSMB will provide details of any items that require urgent action as well as any recommendations made by the iDSMB and will be provided to the principle investigator and the TSC and notified to other participating organizations as well as to the IRB/IEC/HRECs involved.

7.4 Trial Management Trial Management Committee

A Trial Management Committee (TMC) that includes the FOR*Ma*T Trial PI, the trial project managers, the senior trial pharmacist, and appropriate representatives across all the key areas including key clinical with representatives from each participating country in which sites are open, microbiology and laboratory, pharmacology, radiology, statistics and health economics will oversee the logistic running of the trial. To facilitate and ensure adequate communication across the large number of team members and a wide geographic spread, an electronic sharing platform will be established on the study database. A brief template will be required for progress updates and concerns to be addressed weekly from each of the areas. The TMC will report to the TSC. Day-to-day management will be overseen by the FOR*Ma*T Trial PI, the trial project managers, the senior pharmacist and the core trial clinical lead physicians. A Terms of Reference (see Appendix D) will outline the roles and responsibilities of the TMC members.

7.5 FOR*Ma*T Pharmacovigilance Team

The FOR*Ma*T Pharmacovigilance Team will be based in Brisbane, Queensland. All SAEs will be reviewed by the FOR*Ma*T Pharmacovigilance Team and coded in accordance with the CTCAE, version 5.0. Where toxicity thresholds corresponding to CTCAE Grades 3 or higher are reached, critical alerts will be generated and sent to the site investigator who will be required to provide an adverse event report with attribution of causality and the plan for study intervention interruption/withdrawal and further monitoring within 24 hours. Members of the FOR*Ma*T Pharmacovigilance Team include:

- Senior Clinical Pharmacist (Lead),
- FOR*Ma*T Trial database representative,
- Statistician,
- Non-independent senior paediatric physician,
- Non-independent senior adult physician.

7.6 Drug Selection Committee (DSC)

The Trial Management Committee will appoint the DSC which will include a representative amongst the investigators from each participating country as international sites open for recruitment.

The role of the DSC includes assessment of new therapies to be considered for inclusion in a new intervention program.

The DSC will provide a report to be considered by the Trial Statistics team, the TSC and iDSMB and if approved will lead to a new therapy program being included.

7.7 Site Monitoring

The Sponsor and its delegates will have overall responsibility for monitoring. A SIV will occur prior to sites commencing the recruitment of trial participants. The SIV will include assessment of local approval documentation, training of investigators and coordinators at the site with regards to the Master Protocol, procedures and SOPs, data entry and safety reporting, as well as ensuring all investigators and coordinators are trained in Good Clinical Practice (GCP). Monitoring visits will ensure that the study is being conducted according with GCP, that study participants safety, rights and well-being are being protected, the study is being conducted according to the Master Protocol and SOPs are being followed, as well as accurate data entry that is verifiable from source documentation. These will be conducted using both on-site and remote monitoring at intervals specified in the FOR*Ma*T Trial Monitoring Plan and/or Standard Operating Procedure.

If in-person monitoring is not possible, then remote, central and/or local monitoring can be undertaken. Remote monitoring may be used for the Sponsor or delegate to view site files remotely using approved secure systems. Central monitoring of the FOR*Ma*T REDCap Database may be performed by the FOR*Ma*T Trial Management Team. Additionally, the Sponsor may appoint a local delegate who is independent of the site trial team with appropriate trial training and GCP training to monitor the trial site.

8 ETHICAL CONSIDERATIONS AND ADMINISTRATIVE CONSIDERATIONS

The trial will be conducted according to the Declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) and with the laws and regulations of the country in which the research is conducted, whichever represents the greater protection of the individual.

8.1 Research Ethics Approval and Site-Specific Governance

The FOR*Ma*T Master Protocol and Appendices, the participant information and consent form and trial questionnaires will be reviewed and approved by the relevant IRB/IEC/HREC for each participating centre prior to trial initiation. The approved delegate at each site will keep the IRB/IEC/HREC informed as to the progress of the trial and comply with annual reporting requirements. Any documents that the IRB/IEC/HREC may need to fulfil its responsibilities will be submitted to the IRB/IEC/HREC. The IRB/IEC/HREC s written unconditional approval of the FOR*Ma*T Master Protocol and Appendices and associated documents will be obtained prior to study initiation.

The IRB/IEC/HREC must approve any revisions to FOR*Ma*T trial documents, be informed of any serious and/or unexpected AEs occurring during the trial in accordance with the SOPs and policies of the IRB/IEC/HREC and of any new information that may adversely affect the safety of the participants or the conduct of the trial. Reporting requirements are to be adhered to in accordance with local IRB/IEC/HREC and additional site-specific requirements.

8.1.1 FOR*Ma*T Master Protocol and Trial Document Amendments

Any modifications to the approved IRB/IEC/HREC FOR*Ma*T trial documents may not be initiated without prior written IRB/IEC/HREC approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the trial. Such modifications will be submitted to the IRB/IEC/HREC and written verification that the modification was submitted and subsequently approved should be obtained before the updated version is implemented.

8.1.2 Protocol Deviations

A protocol deviation occurs when the Investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant safety and primary endpoint criteria. Examples of protocol deviations may include inappropriate documentation of the informed consent document, implementation of unapproved recruitment procedures, study visits outside of set windows and missing tests and/or laboratory values. The site Investigator, in conjunction with the PI will determine if a protocol deviation will result in participant withdrawal or where relevant, treatment discontinuation. Participants enrolled in the intervention cohort where protocol deviations have occurred involving modifications to the study drug regimen are able to continue to be enrolled and assessed for the remainder of the study.

When a protocol deviation occurs, it will be discussed with the Investigator and a Protocol Deviation Form detailing the deviation will be generated. This form will be signed by the Investigator. A copy of the form will be filed in the investigator site file, and details of the deviation with be entered onto the study database.

Protocol deviations may be minor or major. Minor protocol deviations do not carry significant ethical or administrative consequences. Major protocol deviations are those that affect participant's rights, safety or wellbeing and/or accuracy and reliability of the study data.

Examples of minor protocol deviations:

- Visit non-compliance (for example, study visit is conducted outside of the required timeframe or a procedure is missed) and there are no safety concerns;
- Incorrect execution of the consent form (for example, participant did not date their signature);
- Participant declines to complete scheduled research activities.

Examples of major protocol deviations:

- Use of unapproved recruitment procedures;
- Randomisation of an ineligible participant;
- Use of an unapproved version of the PICF;
- Visit non-compliance (e.g. study visit is conducted outside of the required timeframe or a participant monitoring visit is missed) and there are safety concerns;
- Loss of laptop computer that contained identifiable information about participants;
- Incorrect execution of obtaining consent (for example, consent was not obtained following ICH-GCP).

8.1.2.1 Reporting Requirements

Minor protocol deviations do not need to be reported to the lead IRB/IEB/HREC at the time they occur. All minor deviations must be recorded in the protocol deviation log (paper or electronic versions) and reported to the Sponsor (if applicable).

Major protocol deviations must be reported to the lead IRB/IEC/HREC and to the Sponsor (if applicable) as per local requirements and a protocol deviation form detailing the event completed. This form is required to be signed by the site Investigator and a copy sent to the FOR*Ma*T Trial Management Team.

All protocol deviations (major or minor) must be recorded in a protocol deviation log and submitted to the lead IRB/IEC/HREC as per local requirements. at the time of the annual progress report.

8.2 Confidentiality

The Investigator must ensure that a participant's anonymity will be respected throughout the study and that their identities are protected from unauthorized parties. A participant's privacy and confidentiality will be maintained by the assignment of a unique identification number. On CRF's and other documents submitted to review committees and the Sponsor, participants should not be identified by their names, rather their unique identification number. These numbers will be used to collect, store and report

participant information, including in the trial database. The Investigator should keep a Subject Enrolment Log showing codes, names and dates of birth of the participants in the Investigator Site File. Confidentiality and protection of data will be maintained according to local regulatory requirements. All information disclosed or obtained during the course of the trial are confidential. The Investigator and any person under his/her authority must maintain this confidentiality and must not disclose the information to any third party without the prior written approval of the Investigator.

8.3 Site Reimbursement

Payment to trial sites will be made on a per patient basis: at each of the following time points;

1. Screening;
2. End of intensive for Intervention Participants or week 6 for Observational Cohort participants;
3. End of consolidation for Intervention Program participants or week 52 for Observational Cohort participants;
4. Final timepoint.

Payments will be paid for each time point once the data is entered into the trial database and all queries finalised. Invoices should be prepared at a minimum of every 6 months.

8.4 Data Sharing

All de-identified raw data measured during the trial will be made available on request to relevant regulatory authorities, recognised academic institutions and clinical teams. Data requests must be made in writing with a proposal for data usage to the PI. Upon the approval for data sharing by the PI the requester will be required to sign a data agreement.

8.5 Publication Policy

The results of this trial will be published and/or presented at scientific meetings. The preparation and submittal for publication of manuscripts containing the trial results shall only be done if prior consent is obtained by the PI. Any manuscript requests can be submitted to the FOR*Ma*T Trial Management Team.

APPENDIX A: INTERVENTION PROGRAM

Appendix A describes each Intervention program which includes randomisation to intensive and consolidation interventions and all the relevant detailed statistical methods and simulations.

Appendix A1, describes the first iteration of the intervention program and if new interventions are added either for either the intensive or consolidation phases these would be designated A2, A3 etc. Data from previous programs using the same intervention combinations may be incorporated in the analysis of a new program. Thus, trial data from Appendix A1 could be combined with data from Appendix A2 for example.

Each program in Appendix A will stipulate which of the Discovery studies and Registry linkages described in each Appendix is applicable.

Each Intervention program in Appendix A has the following structure:

1. A statement describing the primary and secondary objectives.
2. A description of the intervention trial design including any specific inclusion or exclusion criteria specific to the intervention program.
3. A description of the interventions and dosing being compared.
4. The methods for assigning treatment arms to the intervention program participants.
5. Additional consent requirements (if required).
6. Specific intervention program trial procedures and schedule of assessments.
7. A description of sub-studies with specific statistical analysis.
8. Cost effectiveness methodology and analysis.
9. A description of statistical analyses and simulations.
10. The Discovery studies applicable to the intervention program (if relevant).

APPENDIX A1: COMPARISON OF STANDARD INTERVENTIONS

1 INTRODUCTION

Appendix A1 describes the initial MABS-PD intervention platform for the FOR*Ma*T trial. Nested within Appendix A1 are sub-studies which are governed by the trial design and conduct described below.

2 PRIMARY OBJECTIVE

To determine the probability of microbiological clearance with acceptable toxicity for treatment combinations tested in FOR*M*aT inclusive of both intensive and consolidation phases for patients with MABS-PD.

The probability of microbiological clearance with acceptable toxicity of treatment combinations will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies (*M. a. abscessus/M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense)* and those with constitutive macrolide resistance and those with mixed NTM infections).

The best therapy combinations may then form the control arms for new intervention studies which can be added as new arms within Appendix A.

The primary outcome for Appendix A1 is MABS clearance from respiratory samples with tolerance at 56 weeks for participants who received short intensive and at 62 weeks for participants who received prolonged intensive.

Definition of tolerance:

Tolerance is based on the Common Terminology Criteria for Adverse Events (CTCAE version 5.0). Only adverse events that are attributed as either possibly, probably or definitely related to study drug will be assessed in the determination of tolerance. “Good” tolerance is defined as no adverse events occurring or only adverse events coded as CTCAE grades 1 and 2. “Poor” tolerance is defined as any adverse events attributed as possibly, probably or definitely related to study drug coded as CTCAE grades 3, 4, or 5.

MABS clearance at final outcome will be defined as;

Negative MABS cultures from four consecutive sputum samples with one of those sputum specimens collected four weeks after the completion of consolidation therapy (either week 56 or 62, depending on treatment arm randomisation). Or, a MABS negative BAL collected four weeks after completion of consolidation (either week 56 or 62, depending on treatment arm randomisation).

3 SECONDARY OBJECTIVES

1- To examine the probability of microbiological clearance at final outcome (irrespective of toxicity) for participants according to treatment path.
2- To describe the safety of the treatment combinations in patients with MABS.
3- To examine the change in FEV_1 z-score at final outcome compared with Day 0 in patients who do and who do not clear MABS at final outcome.
4- To phenotype the structural abnormalities of chest CTs of MABS patients and examine changes in chest CT scores (bronchiectasis, trapped air, % disease) between final outcome and Day 0 (screening) between those who clear and those who do not clear MABS at final outcome.
5- To examine the predictive value of structural abnormalities at Day 0 (screening) CTs for sputum conversion and for progression of structural changes in relation to therapy.
6- To examine the change in HRQoL (CFQ-R respiratory domain) for participants with CF) at final outcome compared with Day 0 according to treatment path and in those that do and those that do not clear MABS.
7- To examine general HRQoL (SF-36 Adults and Peds-QL™ Children) according to treatment path and in those who do and who do not clear MABS at final outcome.
8- To examine the cost effectiveness of the proposed treatment combinations across both intensive and consolidation phases of the trial.

4 APPENDIX A1 DESIGN

Appendix A1 describes the overall trial with combined intensive and consolidation phases (figure 4) and will test therapies that are currently used and are the basis for the current treatment guidelines.

The sub-studies within Appendix A1 i.e. the intensive and consolidation phases of treatment and function as sub-studies nested within the trial:

Sub-study A1.1: Short Intensive Therapy:

A1.1.1: Use of Inhaled Amikacin (IA) During Intensive Therapy to Replace Intravenous Amikacin (IVA) in the Treatment of MABS-PD.

A1.1.2: The Use of Additional Clofazimine to Standard Intravenous Therapies during Intensive Therapy in the Treatment of MABS-PD.

Sub-study A1.2: Duration of Intensive Therapy for Patients with Ongoing Positive MABS cultures After 4 weeks of Intensive Therapy.

Sub-study A1.3: Consolidation Therapy.

Participants enrolled in Appendix A1, will initially be randomised equally to each of three arms (intensive A, B and C) during the intensive phase. At completion of six weeks of intensive therapy, participants with MABS positive isolates (determined from three sputum samples or one BAL collected at week 4±3 days) will be randomised to either an additional six weeks of intensive therapy or randomised to commence consolidation therapy where participants are randomised to one of two treatment arms; consolidation a or b. After the completion of prolonged intensive therapy participants will be randomised to consolidation therapy a or b. Participants who have three MABS negative samples at week six will be randomised at week 6 to consolidation therapy for 46 weeks.

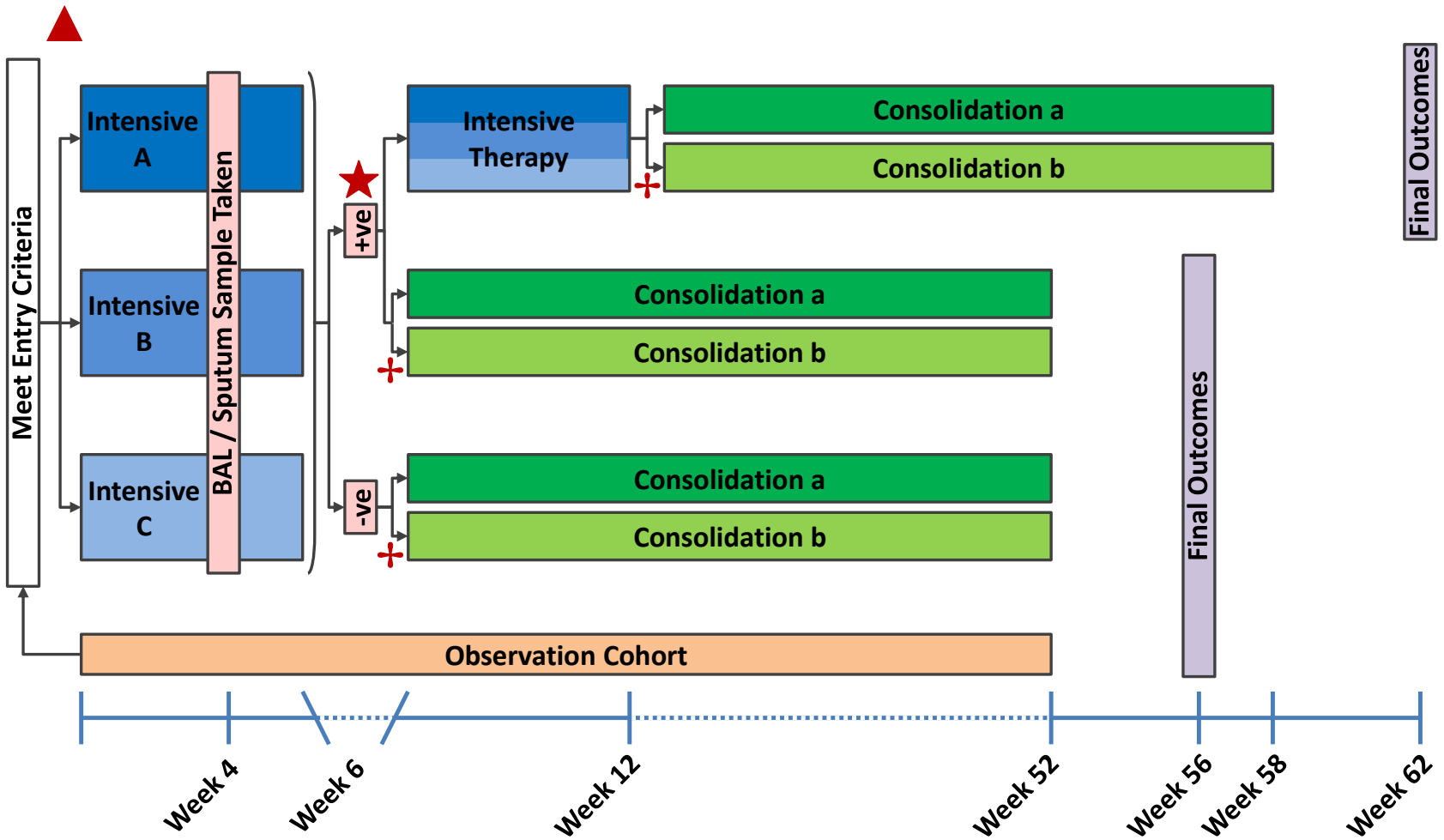

Figure 4: Flow diagram for Appendix A1, Intervention program. Eligibility into the intervention program is determined at screening. At randomisation 1 (▲); participants are randomised between the different intensive therapy arms (Intensive A, Intensive B and Intensive C) for a period of 6 weeks. At the end of intensive therapy, it will be determined if participants are still MABS positive, or MABS negative (cleared). Randomisation 2 (★) will ONLY be for participants who are still MABS positive at week six and will allocate participants to either 1) continue intensive therapy or 2) immediately commence consolidation therapy. Randomisation 3 (✢) allocates participants to the consolidation therapy arms either at week 6 or at week 12 depending on MABS clearance at the end of week 6 and randomisation 2 (if relevant).

5 ELIGIBILITY CRITERIA

Participants are eligible for Appendix A1 if the following criteria are met in addition to those outlined in section 4.3 in the FOR*Ma*T Master Protocol:

5.1 Inclusion Criteria

1. Positive MABS-PD diagnosis meeting all three American Thoracic Society clinical, radiological and microbiological diagnostic criteria for MABS-PD. Defined as;
   a. ***Clinical:*** Pulmonary symptoms and exclusion of other diagnoses.
   b. ***Radiological:*** Nodular or cavitary opacities on chest radiograph or a chest high-resolution computed tomography (HRCT) scan showing multifocal bronchiectasis with multiple small nodules.
   c. ***Microbiological:*** MABS positive culture results from at least two separate expectorated sputum samples.

   or

   Positive culture results from at least one bronchial wash or lavage.

   or

   Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or acid-fast bacilli (AFB)) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washes that are culture positive for NTM.
2. Male or female participants of any age.
3. Informed consent signed by participant or parent/legal guardian if participant is under 18 years of age.
4. Participant has not received MABS-PD treatment in the 12 months preceding assessment of eligibility.
5. Ability to comply with study visits, therapies and study procedures as judged by the site investigator.

5.2 Exclusion Criteria

Participants are ineligible to participate in Appendix A1 if any of the following criteria are met:

- Participants receiving current treatment for MABS, except for participants taking azithromycin as part of routine treatment for CF or bronchiectasis.
- Positive pregnancy test at any time during the FOR*Ma*T trial for females of childbearing potential.
- Breast-feeding.
- An unwillingness to comply with the acceptable methods of contraception, as described below.

- Known hypersensitivity to any of the intervention therapies for which no alternative option(s) have been provided. This includes:
    - Amikacin,
    - Tigecycline,
    - Macrolide antibiotics, and
    - Clofazimine.

5.3 Acceptable Methods of Contraception

The effects of some drugs used during this Intervention trial on the unborn child and on the newborn baby are not known. Because of this, it is important that participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project.

It is therefore important that birth control is used while in this study. Site Investigators should discuss effective methods of contraception with participants. Both male and female participants are to use effective contraception, used correctly with every act of sexual intercourse, from at least 14 days before the first dose of MABS-PD therapy, during the course of the trial and for a period of 90 days after completion of the intervention trial. Acceptable methods of contraception for participants enrolled in Appendix A1 include:

1) Male vasectomy 6 months or more previously, with a documented negative post-vasectomy semen analysis for sperm.
2) Female bilateral tubal ligation performed at least 6 months previously.
3) Female continuous use of an intrauterine device (non-hormone releasing or hormone releasing) for at least 90 days before the first dose of MABS-PD therapy.
4) Female combined (estrogen and progestogen-containing) or progestogen-only oral, injected, implanted or vaginal hormonal contraception associated with inhibition of ovulation.

The barrier contraception methods listed below are not acceptable and are only to be used in special circumstances where the investigator determines that barrier contraception is appropriate.

1) Male and female condom with spermicide (either as a single product if commercially available and/or as allowed according to local regulations; otherwise condom and spermicide as separate products).
2) Male condom with female diaphragm, cervical cap, or vaginal sponge, each with spermicide.

5.3.1 For Female Participants Using Birth Control Pills

The use of birth control pills for contraception must be discussed and approved by the local site Investigator at the start of each phase of the trial and when any changes in therapy occur as, some therapies used during the trial may interact with the effectiveness of birth control pills. Birth control pills should be in successful use from at least 60 days before the first dose of MABS-PD therapy (unless otherwise noted) and until 90 days following the last dose of MABS-PD therapy.

Female participants who change their method of contraception to birth control pills during the trial must continue to use a second form of approved contraception for at least 60 days after starting the use of birth control pills.

5.3.2 For female participants using hormonal injected, implanted or vaginal contraception

Injected, implanted or vaginal hormonal contraception should be used successfully from at least 60 days before the first dose of MABS-PD therapy (unless otherwise noted) and until 90 days following the last dose of MABS-PD therapy.

6 TRIAL CONDUCT

6.1 Informed Consent

In addition to providing consent to the FOR*Ma*T Master Protocol, participant's enrolling in the intervention program are required to sign and date an additional Appendix A1- Intervention program consent form (the Master Appendix A1 Intervention program consent form can be found in Appendix D). Consent to Appendix A1 includes consent to the all Appendix A1 sub-studies. Consent will be obtained from participants or the parent/guardian in accordance with the policies described in section 5.4 of the FOR*Ma*T Master Protocol.

6.2 Pregnancy Information Consent

If a FOR*Ma*T trial participant becomes pregnant or is the biological father of a child conceived while enrolled in this intervention program the site Investigators are requested to provide the participant with the FOR*Ma*T pregnancy information and consent form (Appendix D). The FOR*Ma*T pregnancy and consent form requests to follow the participant and the child for 12 months after conception. Please refer to Appendix E FOR*Ma*T Safety and Monitoring Plan SOP for further detail for safety monitoring and reporting of pregnancies and pregnancy outcomes in female trial participants or female partners who are pregnant to male participants in the intervention program.

6.3 Methods of Assigning Participants to Treatment Arms

Participants will be randomised into different treatment arms for both the intensive and consolidation phase of treatment as described in the Master Protocol section 6.2 using the randomisation technique minimisation, initially in a 1:1:1 ratio for randomisation 1 and 1:1 ratio for randomisations 2 and 3. As described in the Master Protocol, all randomisations will be conducted via REDCap at each trial site by the FOR*Ma*T trial research team. At each stage, study staff will enter the subject demographic data and the stratification factors (see section 6.3 in the Master Protocol) into REDCap, which will then inform them of the participants treatment allocation for that stage.

Following interim analysis, after 100 participants have completed 6 weeks of intensive therapy, we will implement BAR for randomisation 1 to implement the allocation probabilities which will be used until the data support either early stopping for futility, or a maximum sample size is attained.

7 INTENSIVE THERAPY DOSING REGIMEN

At randomisation 1, participants will be randomised to one of three treatment arms during the intensive phase and will receive drug therapy in accordance with the dosing tables below. Drug therapy, administration and duration is dependent on the treatment arm (Intensive A, Intensive B, Intensive C) the participant is randomised to. Drug dosing is based on the participants age (≤17 or ≥18 years of age). The start and end points of each drug therapy, the dose of each drug therapy used, any changes in dosing, as well as all concomitant medications used, will be required to be entered into the CRF.

There are currently three proposed treatment arms in the intensive therapy phase. As described in section 6.2, randomisation 1 dictates the drug therapy that participants will be randomised to. Treatment Arm A is the reference arm (i.e. control).

Intensive Arm A	**Intensive Arm B**	**Intensive Arm C**
1. IV amikacin, and; 2. IV tigecycline, and; 3. IV imipenem or IV cefoxitin, and; 4. Oral azithromycin or oral clarithromycin, and; 5. Clofazimine.	1. Inhaled amikacin, and; 2. IV tigecycline, and; 3. IV imipenem or IV cefoxitin /, and; 4. Oral azithromycin or oral clarithromycin, and; 5. Clofazimine.	1. IV amikacin, and; 2. IV tigecycline, and; 3. IV imipenem or IV cefoxitin, and; 4. Oral azithromycin or oral clarithromycin.

For participants with confirmed mixed NTM infections (slow growers + MABS), ethambutol can be added to the treatment arms (in accordance with the dosing tables below) if required by the treating physician.

The intensive therapy dosing regimen tables outlined below are separated by age (adult, paediatric) and by intensive treatment arm (Arm A, Arm B and Arm C).

Table 3: Intensive therapy dosing regimen for Intensive Arm A in adults

Intensive Arm A: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
IV Amikacin*	15 mg/kg or		Once daily
	20-25 mg/kg		Thrice weekly
*Dosing will be made in accordance with BTS guidelines (48) and is dependent on the physiology, site and TDM outcomes of each participant. In overweight participants use the ideal body weight calculator (Appendix D) or in cases of extremes of actual body weight where body weight is greater than 20% above ideal use the adjusted body weight calculator (Appendix D). To determine ideal body weight for amputees, refer to Appendix D for the table describing the percentage of total weight contributed by individual body parts.			
IV Tigecycline	25 mg increasing by 5 mg every two doses until either maximum dose reached or until patient is unable to tolerate.	50 mg	Twice daily
IV Imipenem/ Cilastatin^ ^ IV Imipenem/cilastatin is preferred but if not tolerated, use IV Cefoxitin **≥50kg Or**	1 g (dose based on imipenem component)*	1 g	Twice – four times daily infused over 1-4 hours as tolerated
*If less than 50kg	15 – 25 mg/kg (dose based on imipenem component)	1 g	Twice – four times daily infused over 1-4 hours as tolerated
IV Cefoxitin^ ^Only for use if imipenem/cilastatin not tolerated	200 mg/kg	4 g	Thrice daily infused over 1-4 hours as tolerated. Or, 12 g over a 24-hour infusion.
Oral Azithromycin* **OR**	250 - 500 mg*#	250 - 500 mg	Once daily

Intensive Arm A: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
*If azithromycin not tolerated, use oral clarithromycin			
# If less than 40kg or poorly tolerated	250 mg	250 mg	Once daily
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	500 mg	500 mg	Twice daily
Oral Clofazimine	100 - 300 mg	100 - 300 mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral Ethambutol* # Dose rounded to the nearest 200mg.	15 mg/kg# or		Once daily
	25 mg/kg#		Thrice weekly
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

Table 4: Intensive therapy dosing regimen for Intensive Arm B in adults

Intensive Arm B: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Inhalation Amikacin (IA) **(IV formulation)**	500 mg	500 mg	Twice daily
IV Tigecycline	25 mg increasing by 5 mg every two doses until either maximum dose reached or until patient is unable to tolerate increasing doses.	50 mg	Twice daily or same total dose over a 24-hour infusion
IV Imipenem/ Cilastatin[^] [^] IV Imipenem/cilastatin is preferred but if not tolerated, use IV Cefoxitin **≥50kg Or**	1 g (dose based on imipenem component)*****	1 g	Twice – four times daily infused over 1-4 hours as tolerated
*If less than 50kg	15 - 25 mg/kg (dose based on imipenem component)	1 g	Twice – four times daily infused over 1-4 hours as tolerated
IV Cefoxitin[^] [^]Only for use if imipenem/cilastatin not tolerated	200 mg/kg	4 g	Thrice daily infused over 1-4 hours as tolerated. Or, 12 g over a 24-hour infusion.
Oral Azithromycin* **OR** *If azithromycin not tolerated, use oral clarithromycin	250 - 500 mg*[#]	250 - 500 mg	Once daily
[#]If less than 40kg or poorly tolerated	250 mg	250 mg	Once daily

Intensive Arm B: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	500 mg	500 mg	Twice daily
Oral Clofazimine	100 - 300 mg	100 - 300 mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral Ethambutol* [#] Dose rounded to the nearest 200mg.	15 mg/kg[#] or		Once daily
	25 mg/kg[#]		Thrice weekly
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

Table 5: Intensive therapy dosing regimen for Intensive Arm C in adults

Intensive Arm C: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
IV Amikacin*	15 mg/kg or		Once daily
	20-25 mg/kg		Thrice weekly
*Dosing will be made in accordance with British Thoracic Society (BTS) guidelines (48) and is dependent on the physiology, site and TDM outcomes of each participant. In overweight participants use the ideal body weight calculator (Appendix D) or in cases of extremes of actual body weight where body weight is greater than 20% above ideal use the adjusted body weight calculator (Appendix D). To determine ideal body weight for amputees, refer to Appendix D for the table describing the percentage of total weight contributed by individual body parts.			
IV Tigecycline	25 mg increasing by 5 mg every two doses until either maximum dose reached or until patient is unable to tolerate.	50 mg	Twice daily or same total dose over a 24-hour infusion
IV Imipenem/ Cilastatin^ ^ IV Imipenem/cilastatin is preferred but if not tolerated, use IV Cefoxitin **≥50kg Or**	1 g (dose based on imipenem component)*	1 g	Twice – four times daily infused over 1-4 hours as tolerated
*If less than 50kg	15 - 25 mg/kg (dose based on imipenem component)	1 g	Twice – four times daily infused over 1-4 hours as tolerated
IV Cefoxitin^ ^Only for use if imipenem/cilastatin not tolerated	200 mg/kg	4 g	Thrice daily infused over 1-4 hours as tolerated. Or, 12 g over a 24-hour infusion
Oral Azithromycin* OR	250 - 500 mg*#	250 - 500 mg	Once daily

Intensive Arm C: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
*If azithromycin not tolerated, use oral clarithromycin			
#If less than 40kg or poorly tolerated	250 mg	250 mg	Once daily
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	500 mg	500 mg	Twice daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral Ethambutol* # Dose rounded to the nearest 200mg.	15 mg/kg# or		Once daily
	25 mg/kg#		Thrice weekly
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

Table 6: Intensive therapy dosing regimen for Intensive Arm A in paediatrics

Intensive Arm A: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
IV Amikacin*		15-30 mg/kg	1500 mg	Once daily
*Dosing will be made in accordance with BTS guidelines (48) and is dependent on the physiology, site and TDM outcomes of each participant. In obese participants use the ideal body weight calculator (Appendix D) or in cases of extremes of actual body weight where body weight is greater than 20% above ideal body weight use the adjusted body weight calculator (Appendix D). To determine ideal body weight or adjusted body weight for amputees, refer to Appendix D.				
IV Tigecycline (ages ≥8 years)	Day 1: (50% of optimal dose)	0.6 mg/kg	25 mg	Twice daily (12 hourly)
	Day 2: (75% of optimal dose)	0.6 mg/kg	25 mg	In the morning
		1.2 mg/kg	50 mg	At night
	Day 3: (100% of optimal dose)	1.2 mg/kg	50 mg	Twice daily (12 hourly)
IV Imipenem/ Cilastatin^ ^ IV Imipenem/cilastatin is preferred but if not tolerated, use IV Cefoxitin **Or**	Day 1-2	15 - 25 mg/kg (dose based on imipenem component)	1 g	Twice daily (12 hourly)
	Day 3	15 - 25 mg/kg (dose based on imipenem component)	1 g	Four times daily (reduce to 3 times daily if not tolerated) (6 or 8 hourly)
IV Cefoxitin^ ^Only for use if imipenem not tolerated		40 mg/kg	2 g	Four times daily (6 hourly)
Oral Azithromycin* **OR** *If azithromycin not tolerated, use oral clarithromycin		10 mg/kg	500 mg	Once daily

Intensive Arm A: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	**Children 1 month – 11 years of age**			
	<8 kg	7.5 mg/kg	62.5 mg	Twice daily
	8-11 kg	62.5 mg	62.5 mg	Twice daily
	12-19 kg	125 mg	125 mg	Twice daily
	20-29 kg	187.5 mg	187.5 mg	Twice daily
	30-40 kg	250 mg	250 mg	Twice daily
	Children 12-18 years of age			
	Dosing independent of weight	500 mg	500 mg	Twice daily
Oral Clofazimine		3-5 mg/kg (rounding of dosing may be used if necessary to account for capsule rounding)	<40kg max 50mg ≥40kg max 100 mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.				
Oral Ethambutol*	20 mg/kg			Once daily
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.				

Table 7: Intensive therapy dosing regimen for Intensive Arm B in paediatrics

Intensive Arm B: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Inhalation Amikacin (IA) (IV formulation)		500 mg	500 mg	Twice daily
IV Tigecycline (ages ≥ 8 years)	Day 1: (50% of optimal dose)	0.6 mg/kg	25 mg	Twice daily (12 hourly)
	Day 2: (75% of optimal dose)	0.6 mg/kg	25 mg	In the morning
		1.2 mg/kg	50 mg	At night
	Day 3: (100% of optimal dose)	1.2 mg/kg	50 mg	Twice daily (12 hourly)
IV Imipenem/ Cilastatin^ ^ IV Imipenem/cilastatin is preferred but if not tolerated, use IV Cefoxitin **Or**	Day 1-2	15 - 25 mg/kg (dose based on imipenem component)	1 g	Twice daily (12 hourly)
	Day 3	15 - 25 mg/kg (dose based on imipenem component)	1 g	Four times daily (reduce to 3 times daily if not tolerated) (6 or 8 hourly)
IV Cefoxitin^ ^Only for use if imipenem/cilastatin not tolerated		40 mg/kg	2 g	Four times daily (6 hourly)
Oral Azithromycin* **OR** *If azithromycin not tolerated, use oral clarithromycin		10 mg/kg	500 mg	Once daily
Oral Clarithromycin*	**Children 1 month – 11 years of age**			
	<8 kg	7.5 mg/kg		Twice daily
	8-11 kg	62.5 mg		Twice daily
	12-19 kg	125 mg		Twice daily
	20-29 kg	187.5 mg		Twice daily

Intensive Arm B: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
*Only for use if azithromycin not tolerated	30-40 kg	250 mg		Twice daily
	Children 12-18 years of age			
	Dosing independent of weight	500 mg	500 mg	Twice daily
Oral Clofazimine		3-5 mg/kg (rounding of dosing may be used where necessary to account for capsule rounding)	<40kg max 50mg ≥40kg max 100 mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.				
Oral Ethambutol*	20 mg/kg			Once daily
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.				

Table 8: Intensive therapy dosing regimen for Intensive Arm C in paediatrics

Intensive Arm C: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
IV Amikacin*		15-30 mg/kg	1500 mg	Once daily
*Dosing will be made in accordance with BTS guidelines (48) and is dependent on the physiology, site and TDM outcomes of each participant. In obese participants use the ideal body weight calculator (Appendix D) or in cases of extremes of actual body weight where body weight is greater than 20% above ideal body weight use the adjusted body weight calculator (Appendix D). To determine ideal body weight or adjusted body weight for amputees, refer to Appendix D.				
IV Tigecycline (ages ≥8 years)	Day 1: (50% of optimal dose)	0.6 mg/kg	25 mg	Twice daily (12 hourly)
	Day 2: (75% of optimal dose)	0.6 mg/kg	25 mg	In the morning
		1.2 mg/kg	50 mg	At night
	Day 3: (100% of optimal dose)	1.2 mg/kg	50 mg	Twice daily (12 hourly)
IV Imipenem/ Cilastatin^ ^ IV Imipenem/cilastatin is preferred but if not tolerated, use IV Cefoxitin **Or**	Day 1-2	15 - 25 mg/kg (dosing based on imipenem component)	1 g	Twice daily (12 hourly)
	Day 3	15 - 25 mg/kg (dosing based on imipenem component)	1 g	Four times daily (reduce to 3 times daily if not tolerated) (6 or 8 hourly)
IV Cefoxitin^ ^Only for use if imipenem/cilastatin not tolerated		40 mg/kg	2 g	Four times daily (6 hourly)
Oral Azithromycin* **OR** *If azithromycin not tolerated, use oral clarithromycin		10 mg/kg	500 mg	Once daily
	Children 1 month – 11 years of age			

Intensive Arm C: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	<8 kg	7.5 mg/kg		Twice daily
	8-11 kg	62.5 mg		Twice daily
	12-19 kg	125 mg		Twice daily
	20-29 kg	187.5 mg		Twice daily
	30-40 kg	250 mg		Twice daily
	Children 12-18 years of age			
	Dosing independent of weight	500 mg	500 mg	Twice daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.				
Oral Ethambutol*	20 mg/kg			Once daily
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.				

7.1 Inhalation Amikacin Guidelines

7.1.1 Nebuliser Type

The 250 mg/mL injection should be used. Two 250mg/ mL ampules (i.e. 500 mg amikacin) should be made up to 4 mL with sodium chloride 0.9%. Prior to administration the patient should receive a bronchodilator, for example, salbutamol, to reduce the risk of coughing and bronchospasm. This can be given nebulised or by using the participants regular inhaler. Amikacin for injection may only be mixed with sodium chloride 0.9%. It must not be nebulised as a mixture with other nebulised drugs (e.g., salbutamol, dornase alfa or other nebulised antibiotics).

High efficiency nebulisers are required to nebulise amikacin (IV formulation). A suitable nebuliser with a filter attachment (eg, SideStream Plus with filter attachment, or Pari LC Plus with filter attachment) are to be used with the nebuliser to prevent environmental deposition of nebulised antibiotics and to reduce the risk of developing antibiotic resistant organisms. Air flow of 6-10 L/min is required to achieve effective nebulisation of amikacin.

7.2 Drug Supply, Storage and Distribution during Intensive Therapy

Supply, distribution and storage of drugs will be made in accordance with site specific guidelines and requirements.

7.3 Amikacin Therapeutic Drug Monitoring

TDM of intravenous amikacin will be required to reduce and monitor toxicity. There is currently variation across trial sites surrounding amikacin TDM methods. To reflect this, acceptable amikacin TDM strategies that can be used in this trial include;

- Trough amikacin levels (independent of minimum inhibitory concentration (MIC) using nomogram for next dose);
- Area under the curve (AUC) target;
- Cmax/MIC and trough;
- AUC/MIC and trough.

The chosen amikacin TDM strategy used must be recorded in the CRF and adhered to consistently for that participant.

For subjects randomized to Intensive Therapy Arm B, a trough amikacin level should be collected although no adjustment of the inhaled amikacin dose should be undertaken.

7.4 Options for Reducing Nausea During Intensive Therapy

Titration of dosing for imipenem and tigecycline is described in tables 3 to 8. Imipenem should also be given over 2-3 hours to reduce nausea. Suggested treatment options that could be considered to reduce nausea are provided in Appendix D.

7.5 Randomisation Two

The second randomisation will ONLY be for participants who are still MABS positive at week six. Randomisation will occur at the end of week 6 and will allocate participants to either;

1- Continue intensive therapy which will be followed by consolidation. Participants will remain on the same intensive therapy drug regimen if randomised to prolonged intensive therapy.
2- Immediately commence consolidation therapy.

8 CONSOLIDATION THERAPY DOSING REGIMEN

Following randomisation 3, participants will receive consolidation treatment in accordance with the dosing tables below. Drug therapy, administration and duration is dependent on the treatment arm (Consolidation Arm a and Consolidation Arm b), age, and/or weight of the participant. The start and end points of each drug therapy, the dose of each drug therapy used, any changes in dosing, as well as all concomitant medications used, will be required to be entered into the CRF.

There are currently two proposed treatment arms that participants will be randomised to during consolidation therapy.

Consolidation Arm a	**Consolidation Arm b**
1. Oral clofazimine, and; 2. Oral azithromycin or oral clarithromycin, and; 3. In combination with one to three of the following oral antibiotics: o Oral linezolid, o Oral trimethoprim/sulfamethoxazole (co-trimoxazole), o Oral doxycycline, o Oral moxifloxacin, o Oral bedaquiline, o Oral rifabutin.	1. Inhaled amikacin 2. Oral clofazimine, and; 3. Oral azithromycin or oral clarithromycin, and; 4. In combination with one to three of the following oral antibiotics: o Oral linezolid, o Oral trimethoprim/sulfamethoxazole (co-trimoxazole), o Oral doxycycline, o Oral moxifloxacin, o Oral bedaquiline, o Oral rifabutin.

For participants with confirmed mixed NTM infections (slow growers + MABS), ethambutol can be added to the treatment arms (in accordance with the dosing tables below) if required by the treating physician.

The intensive therapy dosing regimen tables outlined below are separated by age (adult, paediatric) and by consolidation treatment arm (Arm a and Arm b).

Table 9: Consolidation therapy dosing regimen for Consolidation Arm a in adults

Consolidation Arm a: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Clofazimine	100 – 300 mg	100 – 300 mg	Once daily
Oral Azithromycin* **OR** *Preferred macrolide treatment is azithromycin. If azithromycin not tolerated, use oral clarithromycin.	250 - 500 mg*#	250 - 500mg	Thrice weekly
#If less than 40kg or poorly tolerated	250 mg	250 mg	Thrice weekly
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	500 mg	500 mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.			
Oral Linezolid	600 mg	600 mg	Once daily
Oral Co-trimoxazole (TMP-SMX)	160/800 mg	160/800 mg	Twice daily
Oral Doxycycline	100 mg	100 mg	Once daily
Oral Moxifloxacin	400 mg	400 mg	Once daily
Oral Bedaquiline (weighing at least 30kg)	400 mg	400 mg	Once daily for first 2 weeks
	200mg	200 mg	Thrice weekly for remaining 22 weeks (max duration=6 months)
Oral Rifabutin	5 mg/kg	300 – 450 mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			

Consolidation Arm a: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Ethambutol*	15 mg/kg[#] or		Once daily
[#] Dose rounded to the nearest 200mg.	25 mg/kg[#]		Thrice weekly
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

Table 10: Consolidation therapy dosing regimen for Consolidation Arm b in adults

Consolidation Arm b: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Inhalation Amikacin (IA) (IV formulation)	500 mg	500 mg	Twice daily
Oral Clofazimine	100 – 300 mg	100 – 300 mg	Once daily
Oral Azithromycin* **OR** *Preferred macrolide treatment is azithromycin. If azithromycin not tolerated, use oral clarithromycin.	250 - 500 mg*#	250 -500 mg	Thrice weekly
#If less than 40kg or poorly tolerated	250 mg	250 mg	Thrice weekly
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	500 mg	500 mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.			
Oral Linezolid	600 mg	600 mg	Once daily
Oral Co-trimoxazole (TMP-SMX)	160/800 mg	160/800 mg	Twice daily
Oral Doxycycline	100 mg	100 mg	Once daily
Oral Moxifloxacin	400 mg	400 mg	Once daily
Oral Bedaquiline (weighing at least 30kg)	400 mg	400 mg	Once daily for first 2 weeks
	200 mg	200 mg	Thrice weekly for remaining 22 weeks (max duration=6 months)
Oral Rifabutin	5 mg/kg	300 – 450 mg	Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			

Consolidation Arm b: Adult Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Ethambutol*	15 mg/kg[#] or		Once daily
[#] Dose rounded to the nearest 200mg.	25 mg/kg[#]		Thrice weekly
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

Table 11:Consolidation therapy dosing regimen for Consolidation Arm a in paediatrics

Consolidation Arm a: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Clofazimine		3-5 mg (Dosing may be rounded if needed to account for capsule rounding)	<40kg max 50 mg ≥ 40kg max 100 mg	Once daily
Oral Azithromycin* **OR** *Preferred macrolide treatment is azithromycin. If azithromycin not tolerated, use oral clarithromycin.		10 mg/kg	500 mg	Once daily
Oral* **Clarithromycin** *only for use if azithromycin not tolerated.	**Children 1 month – 11 years of age**			
	<8 kg	7.5 mg/kg		Twice daily
	8-11 kg	62.5 mg		Twice daily
	12-19 kg	125 mg		Twice daily
	20-29 kg	187.5 mg		Twice daily
	30-40 kg	250 mg		Twice daily
	Children 12-18 years of age			
	Dosing independent of weight	500 mg	500 mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.				
Oral Linezolid (ages > 12 years)		600 mg	600 mg	Once daily
Age 1 week – 9 years		10 mg/kg	300 mg	Twice daily
Age > 10 years		10 mg/kg	600 mg	Once daily
Oral Bedaquiline (aged ≥12 years and weighing at least 30kg)		400mg	400mg	Once daily for the first 2 weeks
		200mg	200mg	Three times weekly for remaining 22 weeks (max duration – 6 months)

Consolidation Arm a: Paediatric Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Co-trimoxazole (TMP-SMX)	5 mg TMP/kg	160 mg TMP /800 mg SMX	Twice daily
Oral Doxycycline (ages ≥ 8 years)	2 mg/kg	100 mg	Once daily
Oral Moxifloxacin	10-15 mg/kg (Dose may be rounded to account for capsule rounding where necessary)	400 mg	Once daily
Oral Rifabutin	5 mg/kg		Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral Ethambutol*	20 mg/kg		Once daily
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

Table 12:Consolidation therapy dosing regimen for Consolidation Arm b in paediatrics

Consolidation Arm b: Paediatric Dosing				
Drug		**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Inhalation Amikacin (IA) (IV formulation)		500 mg	500 mg	Twice daily
Oral Clofazimine		3-5 mg/kg (Dosing may be rounded if needed to account for capsule rounding)	<40kg max 50 mg ≥ 40kg max 100 mg	Once daily
Oral Azithromycin* **OR** *Preferred macrolide treatment is azithromycin. If azithromycin not tolerated, use oral clarithromycin.		10 mg/kg	500 mg	Once daily
Oral Clarithromycin* *Only for use if azithromycin not tolerated.	**Child 1 month – 11 years of age**			
	<8 kg	7.5 mg/kg		Twice daily
	8-11 kg	62.5 mg		Twice daily
	12-19 kg	125 mg		Twice daily
	20-29 kg	187.5 mg		Twice daily
	30-40 kg	250 mg		Twice daily
	Child 12-18 years of age			
	Dosing independent of weight	500 mg	500 mg	Twice daily
In combination with one to three of the following oral antibiotics guided by participant susceptibility and tolerance.				
Oral Linezolid (ages > 12 years)		600 mg	600 mg	Once daily
Age 1 week – 9 years		10 mg/kg	300 mg	Twice daily
Age > 10 years		10 mg/kg	600 mg	Once daily
Oral Co-trimoxazole (TMP-SMX)		5 mg TMP/kg	160 mg TMP /800 mg SMX	Twice daily
Oral Doxycycline (ages ≥ 8 years)		2 mg/kg	100 mg	Once daily

Consolidation Arm b: Paediatric Dosing			
Drug	**Recommended Starting Dose**	**Maximum Dose per Dose**	**Frequency of Administration**
Oral Bedaquline (ages ≥12 years and weighing at least 30kg)	400mg	400mg	Once daily for the first 2 weeks
	200mg	200mg	Three times weekly for remaining 22 weeks (max duration – 6 months)
Oral Moxifloxacin	10-15 mg/kg (Dosing may be rounded if needed to account for capsule rounding)	400 mg	Once daily
Oral Rifabutin	5 mg/kg		Once daily
For participants with confirmed mixed NTM (slow growers + MABS) infections, there is an option to add oral ethambutol to the treatment arm in accordance with the dosing below.			
Oral Ethambutol*	20 mg/kg		Once daily
*Ethambutol should be dosed on ideal body weight. Ideal body weight calculator can be found in Appendix D.			

8.1 Drug Supply, Storage and Distribution during Consolidation Therapy

Supply, distribution and storage of drugs will be made in accordance with site specific guidelines and requirements.

8.2 Drug Compliance during Consolidation Therapy

The procedures for distribution of consolidation therapy are site dependent. Drug compliance during consolidation therapy will be captured from pharmacy records of drugs dispensed to the participant while enrolled in FOR*Ma*T Appendix A1.

8.3 Schedule of Assessments Incorporating Core Trial Procedures and Those Required for Intervention Program A1.

In addition to the core trial procedures described in Table 1 of the Master Protocol, participants enrolled in this Intervention program are required to undertake additional trial procedures in accordance with tables 13-15 (see below). The three-different schedules of assessment tables outlined below describe the three different therapy pathways that a participant could be randomised to while enrolled in Appendix A1. Acceptable study visit windows are outlined in tables 13-15. A FOR*Ma*T trial visit date calculator spreadsheet is available to calculate the study visit window for Intervention Program participants.

9 INTERVENTION PROGRAM PROCEDURES AND TOXICOLOGY MONITORING

In addition to the core trial procedures described in the Master Protocol, participants enrolled in the Intervention program are required to undertake procedures and regular toxicology monitoring. The type of toxicology monitoring procedure required will be determined by the treatment arm the participant is allocated to, and in accordance with the schedule of assessments outlined in tables 13-15. Toxicology thresholds will be defined in accordance with CTCAE criteria as outlined in the Master Protocol, section 5.9.2. The outcome of all the assessments below are to be documented in the corresponding CRF and entered into the FOR*Ma*T database.

9.1 Serum/Urine Pregnancy Test

All female participants of childbearing potential are required to undergo regular pregnancy testing while enrolled in the intervention program. A serum pregnancy test will be performed at screening and at the final study visit; either week 56/62 depending on treatment arm allocation and if applicable at the early termination visit. A urine β-hCG test is acceptable for regular pregnancy monitoring from day 1 in accordance with the schedule of assessments outlined in tables 13-15. Both urine and serum pregnancy tests are to be performed in accordance with site specific procedures.

9.2 Audiogram

To monitor aminoglycoside induced ototoxicity, regular audiometry assessments will be undertaken in accordance with the schedule of assessments outlined in tables 13-15. The battery of assessments to be completed should comply with the site-specific requirements. Assessments can be conducted in either a hospital or community setting.

9.3 Vestibular Monitoring

Vestibular toxicity monitoring will document any vestibular symptoms (motion-induced oscillopsia, postural instability and gait unsteadiness) associated with prolonged aminoglycoside use. Assessments will take place in accordance with the schedule of assessments outlined in table 13-15. Testing can be completed either by the treating physician or a physiotherapist. Videos of each of the tests are available on the Investigator only component of the FOR*Ma*T website.

In adults, each testing session will include at a minimum dynamic visual acuity testing and head impulse testing (HIT)/video HIT (VHIT) and the Romberg on foam test if available. In children, only the Head Impulse Test (HIT) will be measured.

9.3.1 Dynamic Visual Acuity Testing

Ask the patient to read a visual acuity chart (e.g. Snellen) while sitting still at recommend distance. This result is the static visual acuity. Repeat task while oscillating the patient's head horizontally or vertically at 1 to 2 Hz. An abnormal DVA is defined as loss of at least three lines of visual acuity compared with static condition (horizontal and/or vertical).

9.3.2 Head Impulse Testing (HIT)

Stand in front of the seated patient and ask the patient to focus on a target directly in front of them (e.g. the examiner's nose). Briskly rotate the patient's head horizontally approximately 10 to 20° amplitude, watching the patient's eyes closely. In normal subjects, the patient's eyes remain still as they remain on target. However, in a patient with impaired vestibulo-ocular reflex (VOR), the patient's eyes drift off the target and require a corrective 'catch-up' saccade to re-fixate on the target and stabilise vision. This catch-up saccade is a small amplitude horizontal eye movement in the opposite direction of the head turn and should occur with every head impulse (repeatable).

If available, video HIT (vHIT) is recommended as this has a higher sensitivity than the traditional bedside head impulse test at detecting impaired VOR. Main benefits include detecting covert (hidden) catch up saccades and peer review.

9.3.3 Romberg on Foam Test

On a foam surface, ask the participant to stand still with two feet together. The participant should be able to stand steady with their eyes open. If the participant is not able to perform this task, ask them to

separate their feet to minimal distance that allows them to do so. Repeat the task this time with their eyes closed. Record if the participant falls (positive Romberg test) or does not (negative Romberg test).

Document vestibular test results and any further actions (if results indicate significant vestibular impairment) in the CRF.

9.4 Physical Examination: Intervention

The physical examination at screening and at final outcome will be performed in accordance with the procedures outlined in the FOR*Ma*T Master Protocol, section 5.5.5. In between these visits, symptom directed physical examinations and symptom directed vital signs can be performed at the discretion of the FOR*Ma*T trial doctor in accordance with the schedule of assessments outlined in tables 13-15.

9.5 Electrocardiogram (ECG)

A standard 12 lead ECG will be performed in accordance with tables 13-15 and site-specific procedures after the participant has been supine for at least 5 minutes. A qualified physician will interpret, sign and date the ECG. The QT interval and the clinical interpretation will be recorded in the CRF and ECGs will be required to be scanned and stored in the CRF.

9.6 Blood Sampling

Blood samples will be collected in accordance with tables 13-15. Liver function, full blood count and chemistry and renal function tests (refer to FOR*Ma*T SOP 15 for specific blood parameters) are to be performed in accordance with the local pathology requirements. Results will be required to be entered into the CRF within a 3-day window.

9.7 Study Medication Review

Participant adherence to MABS-PD treatment in the intervention cohort will be measured via two indirect methods. Self-reported adherence will be assessed using a validated questionnaire, the Medication Adherence Questionnaire (also known as the 8-item Morisky Medication Adherence scale) (49, 50) at the timepoints outlined in Table 1 and results recorded in the Medication Adherence Questionnaire CRF. Data from pharmacy prescription refill records and prescription claims databases will be obtained and used to calculate the refill adherence measure, Medication Possession Ratio (MPR) (51-54).

9.8 Health Related Quality of Life and Utility Measures

HRQoL and utility tools will be collected in accordance with the Master Protocol and tables 13-15. All HRQoL measures must be performed prior to any clinical measurements.

Table 13: Schedule of Assessments for Intensive Therapy

ASSESSMENT	SCREENING	Wk 1 - Wk 6 INTENSIVE THERAPY																			
		INTENSIVE ARM A							INTENSIVE ARM B							INTENSIVE ARM C						
	Day 0	Day 1	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Day 1	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Day 1	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
	-14 Days	Exact	+3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	Exact	+3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	Exact	+3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days
Informed consent for Appendix A1	✓																					
Clinic visit	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Review eligibility	✓																					
Respiratory sample: Sputum/BAL	✓					✓ x3^							✓ x3^							✓ x3^		
Height and Weight	✓	✓	✓	✓		✓		✓	✓	✓	✓		✓		✓	✓	✓	✓		✓		✓
Medication Review	✓	✓		✓		✓		✓	✓		✓		✓		✓	✓		✓		✓		✓
Spirometry	✓							✓							✓							✓
Chest CT	✓																					
Serum/Urine Pregnancy Test and Breastfeeding Status*	✓	✓		✓		✓		✓	✓		✓		✓		✓	✓		✓		✓		✓
Audiogram	✓$					✓							✓							✓		
Vestibular Monitoring	✓					✓							✓							✓		
Chemistry and Renal Function	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓
ECG	✓		✓					✓		✓					✓		✓					✓
Liver Function Tests	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓

ASSESSMENT	SCREENING	Wk 1 - Wk 6																		
		INTENSIVE THERAPY																		
		INTENSIVE ARM A							INTENSIVE ARM B							INTENSIVE ARM C						
	Day 0	Day 1	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Day 1	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Day 1	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
	-14 Days	Exact	+3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	Exact	+3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	Exact	+3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days
Full Blood Count	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓
Physical examination: Intervention	✓	✓						✓	✓						✓	✓						✓
Amikacin Therapeutic Drug Monitoring		✓	✓	✓				✓								✓	✓	✓				✓
Inhaled Amikacin Trough Levels									✓	✓												
EQ-5D-5L [E]	✓							✓							✓							✓
SF36 [E]	✓							✓							✓							✓
PedsQ™ [#]	✓							✓							✓							✓
CFQ-R**	✓							✓							✓							✓
EQ-5D-Y [Q]	✓							✓							✓							✓
Child Health Utility 9D [U]	✓							✓							✓							✓
SGRQ [S]	✓							✓							✓							✓
Medication Adherence Questionnaire[A]						✓							✓							✓		

Abbreviations: Week (wk).

^ To determine MABS clearance following intensive therapy all three sputum samples or one BAL sample are required to be collected in week 4 (±3 days).

* Only required in female participants of childbearing potential. A serum pregnancy test is required at screening. A urine pregnancy test is acceptable at all other times unless otherwise stated.

[$]Screening audiology can be performed if a participant has commenced intensive therapy but must be performed within three (3) days of first receiving treatment.

[E] The EQ-5D-5L and the SF-36, are only to assessed in participants ≥16 years of age.

** The CFQ-R is only to be completed in participants with CF. The age appropriate CFQ-R assessment should be selected; CFQ-R adult/teen, CFQ-R child and CFQ-R parent.

[#] The PedsQL™ is only to be assessed in participants ≤16 years of age. If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R. The age appropriate questionnaire should be selected.

[Q] The EQ-5D-Y is only to be assessed in participants 8-15 years of age.

[U] The Child Health Utility is only to be assessed in participants ≤16 years of age.

[S] The SGRQ is only to be assessed in non-CF participants 18 years of age and older.

[A] Medication Adherence Questionnaire is only to be completed in participants in the intervention cohort on outpatient based MABS-PD treatment.

Table 14: Schedule of Assessments for Participants Randomised to Prolonged Intensive Therapy Followed by Consolidation Therapy and Final Outcome

ASSESSMENT	Wk 7- Wk 12																		Wk 13 - Wk 58				FINAL
	PROLONGED INTENSIVE THERAPY																		DELAYED CONSOLIDATION				
	INTENSIVE ARM A						INTENSIVE ARM B						INTENSIVE ARM C										
	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 18	Wk 28	Wk 38	Wk 58	Wk 62
	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±5 Days	±5 Days	±5 Days	+5 Days	+14 Days
Clinic visit	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Respiratory sample: Sputum/BAL				✓ x3^						✓ x3^						✓ x3^			✓NP	✓NP	✓NP	✓NP	✓BAL
Height and Weight		✓		✓		✓		✓		✓		✓		✓		✓		✓	✓	✓	✓	✓	✓
Medication Review				✓		✓				✓		✓				✓		✓	✓	✓	✓	✓	✓
Spirometry						✓						✓						✓				✓	✓
Chest CT						✓& Optional						✓& Optional						✓& Optional					✓
Serum/Urine Pregnancy Test and Breastfeeding Status*			✓			✓			✓			✓			✓			✓	✓	✓	✓	✓	✓
Audiogram		✓				✓		✓				✓		✓				✓		✓			✓
Vestibular Monitoring		✓				✓		✓				✓		✓				✓		✓			✓
Chemistry and Renal Function	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
ECG						✓						✓						✓		✓			✓
Liver Function Tests	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

ASSESSMENT	Wk 7- Wk 12																		Wk 13 - Wk 58				FINAL
	PROLONGED INTENSIVE THERAPY																		DELAYED CONSOLIDATION				
	INTENSIVE ARM A						INTENSIVE ARM B						INTENSIVE ARM C										
	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 18	Wk 28	Wk 38	Wk 58	Wk 62
	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±5 Days	±5 Days	±5 Days	+5 Days	+14 Days
Full Blood Count	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physical examination: Intervention						✓						✓						✓	✓	✓	✓	✓	✓
Amikacin TDM						✓												✓					
EQ-5D-5L[E]						✓						✓						✓	✓	✓		✓	✓
SF-36 [E]						✓						✓						✓	✓	✓		✓	✓
PedsQL™ [#]						✓						✓						✓	✓	✓		✓	✓
CFQ-R**						✓						✓						✓	✓	✓		✓	✓
EQ-5D-Y [Q]						✓						✓						✓	✓	✓		✓	✓
Child Health Utility 9D [U]						✓						✓						✓	✓	✓		✓	✓
SGRQ [S]						✓						✓						✓	✓	✓		✓	✓
Medication Adherence Questionnaire[A]				✓						✓						✓			✓	✓	✓	✓	

Abbreviations: Week (Wk), Final outcome (final).

^ To determine MABS clearance all three sputum samples or one BAL sample are required to be collected in week 10 (±3 days).

[NP] Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.

[BAL] Participants who have produced sputum samples during Weeks 18, 28, 38 and 52/58 are requested to provide three additional sputum samples collected at least one week apart nearer to the end of Weeks 52/58. For participants who were unproductive (intermittent or continual) during Weeks 18, 28, 38 and 52/58,

a BAL sample is to be collected during Week 56 (for observational participants and short intensive participants) or Week 62 (for prolonged intensive participants).[&]Week 12 CT Scan is optional and requires participant to consent to FOR*Ma*T Sub-Study B3: Imaging.

*Only required in female participants of childbearing potential. A urine pregnancy test is acceptable at all times except for at the final outcome when a serum pregnancy test will be measured.

[E] The EQ-5D-5L, SF-36, are only to assessed in participants ≥16 years of age.

**The CFQ-R is only to be completed in participants with CF. The age appropriate CFQ-R assessment should be selected; CFQ-R adult/teen, CFQ-R child and CFQ-R parent.

[#] The PedsQL™ is only to be assessed in participants ≤16 years of age. If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R. The age appropriate questionnaire should be selected.

[Q] The EQ-5D-Y is only to be assessed in participants 8-15 years of age.

[U] The Child Health Utility is only to be assessed in participants ≤16 years of age.

[S] The SGRQ is only to be assessed in non-CF participants 18 years of age and older.

[A] Medication Adherence Questionnaire is only to be completed in participants in the intervention cohort on outpatient based MABS-PD treatment.

Table 15: Schedule of Assessments for Participants Randomised to Immediate Consolidation Following Intensive Therapy and Final Outcome

ASSESSMENT	Wk 7 - Wk 52 IMMEDIATE CONSOLIDATION						FINAL
	Wk 10 ±3 Days	Wk 12 ±3 Days	Wk 18 ±5 Days	Wk 28 ±5 Days	Wk 38 ±5 Days	Wk 52 +5 Days	Wk 56 +14 Days
Clinic visit	✓	✓	✓	✓	✓	✓	✓
Respiratory sample: Sputum/BAL	✓ x3^		✓NP	✓NP	✓NP	✓NP	✓BAL
Height and Weight	✓	✓	✓	✓	✓	✓	✓
Medication Review	✓	✓	✓	✓	✓	✓	✓
Spirometry		✓				✓	✓
Chest CT		✓& Optional					✓
Serum/Urine Pregnancy Test and Breastfeeding Status*		✓	✓	✓	✓	✓	✓
Audiogram		✓		✓			✓
Vestibular Monitoring		✓		✓			✓
Chemistry and Renal Function	✓	✓	✓	✓	✓	✓	✓
ECG		✓		✓			✓
Liver Function Tests	✓	✓	✓	✓	✓	✓	✓
Full Blood Count	✓	✓	✓	✓	✓	✓	✓
Physical examination: Intervention		✓	✓	✓	✓	✓	✓
EQ-5D-5L [E]		✓	✓	✓		✓	✓
SF-36 [E]		✓	✓	✓		✓	✓
PedsQL™ #		✓	✓	✓		✓	✓
CFQ-R**		✓	✓	✓		✓	✓
EQ-5D-Y [Q]		✓	✓	✓		✓	✓
Child Health Utility 9D [U]		✓	✓	✓		✓	✓
SGRQ [S]		✓	✓	✓		✓	✓
Medication Adherence Questionnaire[A]	✓		✓	✓	✓	✓	

Abbreviations: Week (Wk), Final outcome (final).

^ Three sputum samples or one BAL sample are required to be collected in week 10 (±3 days).

[NP] Participants unable to produce a sputum sample (expectorated or induced) to be marked as unproductive on the CRF.

[BAL] Participants who have produced sputum samples during Weeks 18, 28, 38 and 52/58 are requested to provide three additional sputum samples collected at least one week apart nearer to the end of Weeks 52/58. For participants who were unproductive (intermittent or continual) during Weeks 18, 28, 38 and 52/58, a BAL sample is to be collected during Week 56 (for observational participants and short intensive participants) or Week 62 (for prolonged intensive participants).

[&]Week 12 CT Scan is optional and requires participant to consent to FOR*Ma*T Sub-Study B3: Imaging.

* Only required in female participants of child bearing potential. A urine pregnancy test is acceptable at all times except for at the final outcome when a serum pregnancy test will be measured.

[E] The EQ-5D-5L and the SF-36, are only to assessed in participants ≥16 years of age.

[#] If both the PedsQL™ and the CFQ-R are administered where possible, the PedsQL™ should be administered prior to the CFQ-R. The age appropriate questionnaire should be selected.

**Select the age appropriate CFQ-R assessment; CFQ-R adult/teen, CFQ-R child and CFQ-R parent.

[Q] The EQ-5D-Y is only to be assessed in participants 8-15 years of age.

[U] The Child Health Utility is only to be assessed in participants ≤16 years of age

[S] The SGRQ is only to be assessed in non-CF participants 18 years of age and older.

[A] Medication Adherence Questionnaire is only to be completed in participants in the intervention cohort on outpatient based MABS-PD treatment.

10 COST-EFFECTIVENESS AND RESOURCE UTILISATION METHODOLOGY

10.1 Introduction

Of the different NTM infections, MABS-PD is reported to have the highest associated treatment costs. To help inform health policy about the treatment of MABS-PD the healthcare costs and cost-effectiveness of therapies are required.

To capture cost utilisation data in the treatment of MABS-PD each site will be required to enter the complete resource utilisation data set in a minimum 20% of participants enrolled in Appendix A1. These data will be entered in the FOR*Ma*T REDCap database by the site FOR*Ma*T research team members. All other participants will require the minimum resource utilisation data set to be entered in the FOR*Ma*T REDCap database. Minimum data requirements will be flagged in the FOR*Ma*T REDCap database and research staff will not be able to continue entering data unless these data are entered.

A health resource utilisation and cost questionnaire will be completed by all consenting FOR*Ma*T participants with respect to both the intensive and consolidation phases of the intervention trial. The survey will be administered at the same tine points as the EQ-5D and CHU9D surveys (approximately every six weeks). The questionnaire is based on the 'Annotated cost questionnaire' originally designed for the UK setting but will be appropriately adapted for each country. Piloting of the survey will be completed prior to implementation.

10.2 Cost-Effectiveness Analysis

Cost-effectiveness analysis will be conducted using a cost-utility analysis for each of the participating countries health system perspective based on aggregate trial participant data. The summary health outcome measure to be included in the analysis is quality adjusted life years (QALYs). QALYs are estimated based on the sum of the duration of time living in respective health states. In this analysis individual QALYs will be estimated using an area under the curve approach. Data on participants health state will be informed by the EQ-5D-5L and CHU9D for adults and children respectively. The utility values for each health state will be informed by country specific utility valuation sets and applied respectively for each country specific analysis.

Costs will include both medication and diagnostic costs associated with treatment and monitoring as well as costs associated with community, hospital and emergency department presentations during treatment phase. This will enable the inclusion of costs associated with treatment related adverse events.

Descriptive data for treatment related and all other health care utilisation will be presented with respect to counts at an aggregated descriptor level. For example, ICD-10 codes for admitted patient separations,

anatomical therapeutic chemical classification for prescription medications, major diagnostic classification for emergency department presentations.

Comparative analysis will be completed on an intention-to-treat strategy where patients are analysed within the treatment group to which they were randomly allocated, regardless of, for example, whether or not they received the treatment medication they were allocated to. Results will be presented as incremental cost-effectiveness ratios (ICERs) with their corresponding 95% confidence intervals and cost-effectiveness acceptability curves (CEACs). The ICER represents the additional cost for an incremental improvement in outcome when comparing between groups (e.g. experimental group 1 (exp) to comparator group 2 (com):

$$ICER=(Cost_{exp}-Cost_{comp}) / (Effect_{exp}-Effect_{com})$$

All current treatment paths within the platform trial will be evaluated simultaneously prior to adjustment in the platform trial design. Primary analysis (i) will be with respect to which therapy combination (including both the initial and consolidation phases) is most cost-effective. Additional analyses will also consider which therapy combination is the most cost-effective with respect to initial treatment (ii); which therapy combination is the most cost-effective for those who are clear at 4 weeks (iii); and which therapy combination is the most cost-effective for those who have remained positive at 4 weeks (iv). Cost effectiveness analyses within each of the iv analysis sets will be completed simultaneously with ICER results presented relative to the treatment group with the fewest estimated mean QALYs. An intervention arm will be considered cost-effective where the ICER is less than or equal to each countries respective cost effectiveness threshold, the value of an incremental improvement in quality adjusted life years. However, there is considerable uncertainty as to any given countries λ. CEACs will therefore be presented as a way to examine the probability of the intervention being cost effective, given various values of a countries λ.

As the ICER is a ratio, several difficulties may arise in its interpretation. A positive ratio can represent both higher costs and better outcomes in the experimental group, and lower costs and less favourable outcomes (compared to the control group). Moreover, a negative ratio can indicate higher costs coupled with worse outcomes (comparator dominates the intervention) as well as lower costs and better outcomes (intervention dominates the comparator). Scatter plots of at least 1000 bootstrap replications of the ICER will therefore be presented to aid the interpretation of the ICER and the assessment of uncertainty surrounding it. Moreover, the net monetary benefit (NMB) of each intervention will also be estimated. Whereby, net benefit is estimated by multiplying the outcome by λ, and subtracting incremental costs.

10.3 Cost Utility Analysis

Analysis of the cost-utility of the interventions will be conducted with the use of a Seemingly Unrelated Regression (55). The system of Seemingly Unrelated Regression equations is being used as it has been argued that such methods are generally robust to skewed data and to allow for any correlation between costs and effects. Here, separate regression models will be fitted for a) the entire population b) block-randomised sub-groups. Within these analytical frameworks it is possible to control for baseline scores, and confounding variables (56, 57). Bootstrapping will be used to obtain multiple estimates for the group difference, allowing a probabilistic interpretation of results: The percentage of estimates of the group difference greater than one – indicating better results for the experimental group – is plotted for each value of WTP, resulting in the CEAC (58).

All models will take into account clustering by site. Analyses will be shown for a) health system costs only and b) costs including direct and indirect health care costs including for example, patient/carer productivity losses. Missing data will be addressed using multiple imputation (59). A blinded data review will inform the imputation strategy and the selection of multivariable models. To assess the effect of missing data, economic analyses will be performed on the imputed data, and on complete cases. A complete-case analysis will only include participants if complete cost and outcome data at all time points are available for that participant.

11 STATISTICAL ANALYSIS AND SIMULATIONS FOR APPENDIX

11.1 Simulation Descriptions

We have powered the trial assuming that 300 Intervention Program trial participants are recruited in total. Two interim analyses to update the allocation probabilities using Bayesian Adaptive Randomisation are conducted after 100 and 200 patients have six-week information collected.

We have considered six different scenarios for the probability of clearance with tolerability outcome at the three different randomisations. Tables 17-19 show these probabilities for each scenario.

Table 16: Intensive phase six-week outcome probabilities

Arm	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
A: IVA with clofazimine (reference arm)	0.5	0.45	0.45	0.5	0.7	0.50
B: IA with clofazimine	0.5	0.65	0.45	0.7	0.5	0.65
C: IVA without clofazimine	0.5	0.45	0.65	0.7	0.5	0.65

Table 17: Continued intensive vs not 12-week outcome probabilities

Pathway	12week 1	12week 2	12week 3	12week 4	12week 5	12week 6
1. Positive, Intensive, Oral only	0.5	0.5	0.7	0.5	0.5	0.30
2. Positive, Intensive, Oral+IA	0.5	0.5	0.7	0.5	0.5	0.30
3. Positive, No intensive, Oral only	0.5	0.7	0.5	0.6	0.4	0.55
4. Positive, No intensive, Oral+IA	0.5	0.7	0.5	0.6	0.4	0.55

Table 18: Consolidation phase long-term outcome probabilities

Pathway	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
1. Positive, Intensive, Oral only	0.5	0.5	0.30	0.7	0.60	0.3
2. Positive, Intensive, Oral+IA	0.5	0.7	0.45	0.5	0.80	0.5
3. Positive, No intensive, Oral only	0.5	0.5	0.30	0.7	0.55	0.5
4. Positive, No intensive, Oral+IA	0.5	0.7	0.45	0.5	0.75	0.7
5. Negative, Oral only	0.5	0.5	0.30	0.7	0.75	0.7
6. Negative, Oral+IA	0.5	0.7	0.45	0.5	0.95	0.9

11.2 Scenario Definitions

Scenario 1 represents the null scenario where all treatment strategies have the same effect.

Scenario 2 represents a scenario where one intensive phase treatment provides an advantage in probability of clearance with tolerability, continued intensive is worse than not at 12 weeks, and oral+IA provides an advantage over oral for consolidation for each type of patient.

Scenario 3 represents a similar scenario to Scenario 2 except continued intensive is better than not continued intensive at 12 weeks and consolidation probabilities are lower.

In Scenario 4, both arm B and C provide advantages over arm A at 6 weeks, with oral being superior to oral+IA at the long-term outcome, not continuing intensive is better than continuing at 12 weeks with lower probabilities than in Scenario 2.

In Scenario 5, arm A is superior to B and C. Consolidation probabilities are highly variable by participant pathway (albeit with oral+IA having the same absolute improvement in each case), continued intensive is better than not at 12 weeks with a lower probability of clearance with tolerability.

Scenario 6 is similar to Scenario 5, with each pathway having generally lower probability of clearance with tolerability at the long-term outcome, both arm B and C provide advantages over arm A at 6 weeks with lower probability of clearance with tolerability and continued intensive is worse than not at 12 weeks with lower probabilities than in Scenarios 2 and 4.

In each scenario, 100000 simulation replicates are conducted. In each replicate, 300 patients have their pathways through the trial simulated with 6-week outcome, 12-week outcome and long-term outcome generated with a binomial distribution with probability according to the relevant scenario in the above tables.

The intensive phase uses BAR. For the first 100 patients, patients are randomised equally (1:1:1) between the three arms. At this point, and subsequently after 200 patients have been recruited, the allocation probabilities are updated according to the BAR procedure described below.

Let p_A, p_B and p_C represent the true probability of six-week clearance with tolerability on arms A, B and C respectively. At each of the interim analyses the posterior probabilities of: 1) IA being superior to IVA with clofazimine, $P(p_B > p_A)$ and 2) IVA without clofazimine being superior to IVA with clofazimine, $P(p_C > p_A)$, are calculated. These are then used to set the allocation ratio for randomising the next stage of patients respectively to arms A, B and C namely π_A, π_B, π_C. The formula used to set the allocation is:

$$\pi_A = 1/3$$
$$\pi_B = (2/3) \times \frac{P(p_B > p_A)^{\gamma(n/N)}}{P(p_B > p_A)^{\gamma(n/N)} + P(p_C > p_A)^{\gamma(n/N)}}$$
$$\pi_C = (2/3) \times \frac{P(p_C > p_A)^{\gamma(n/N)}}{P(p_B > p_A)^{\gamma(n/N)} + P(p_C > p_A)^{\gamma(n/N)}},$$

where $\gamma(n/N) = 12 * (\left(\frac{n}{N}\right))^{2.5}$, n/N represents the proportion of planned sample size recruited so far (1/3 at first interim and 2/3 at second interim). This procedure follows the one recommended by Wason and Trippa (60).

11.3 Intensive Phase Power Simulations

11.3.1 Intensive Phase Power

The intensive phase data is analysed (in simulations) with a test of proportions assuming a normal approximation. Table 20 shows the power of the trial for testing each research question in the different scenarios.

Table 19: Intensive phase outcome power for different hypotheses, 100000 simulation replicates

Comparison	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
B vs A	0.060	0.870	0.048	0.81	0.87	0.56
C vs A	0.059	0.052	0.870	0.81	0.86	0.56

Generally, for cases where there is >=20% difference between arms, the trial is well powered. The AR procedure is advantageous when one experimental arm provides an advantage but not the other (as then more patients are randomised to the effective arm).

11.3.2 Prolonged Intensive vs Not prolonged at 12 weeks Power

Table 21 shows the power for the design to detect significant improvements at 12 weeks from randomising non-cleared patients between prolonged intensive and not continuing intensive.

In this case, we investigated different ways of analysing the data to allow adjustments for the study stage and treatment groups.

Table 20: 12 weeks outcome power for different hypotheses, 100,000 simulation replicates

Pathway	12week1	12week2	12week3	12week4	12week5	12week6
Continued intensive vs not, 12 weeks	0.052	0.67	0.67	0.18	0.21	0.80
Model1	0.054	0.67	0.67	0.19	0.21	0.80
Model1 red	0.053	0.67	0.67	0.19	0.21	0.80
Oral vs intensive	0.049	0.48	0.50	0.13	0.15	0.62
Oral+IA vs Intensive	0.051	0.48	0.50	0.13	0.15	0.62
Model2	0.055	0.57	0.57	0.15	0.17	0.71
Model2 red	0.053	0.57	0.56	0.15	0.17	0.71

Where:

Model 1: $logit(P = 1|treat, stage) = \alpha_0 + \alpha_1\, treat + \alpha_2 stage_2 + \alpha_3 stage_3$

Treat=0 if a patient with a positive sputum (after six weeks) had an extended intensive treatment.

Treat=1 if a patient with a positive sputum started the oral consolidation directly (either oral consolidation alone or plus IA). Stage corresponds to the three recruitment periods. $\boldsymbol{Stage_2} = 1$ if a patient was recruited during the second period, $\boldsymbol{Stage_3} = \mathbf{1}$ if a patient was recruited during the third period, and $\boldsymbol{Stage_2} = \boldsymbol{Stage_3} = \mathbf{0}$ if a patient was recruited during the first period.

Model 2: $logit(P = 1|path, stage) = \alpha_0 + \alpha_1\, path_2 + \alpha_2 path_3 + \alpha_3 stage_2 + \alpha_4 stage_3$, where path corresponds to one of the three pathways followed by each patient after six weeks. P$\mathbf{ath_2} = 1$ if a patient with a positive sputum after six weeks started an oral consolidation only, $\mathbf{path_3} = 1$ if a patient with a positive sputum after six weeks started an oral consolidation plus IA, and P$\mathbf{ath_2} =$ P$\mathbf{ath_3} = 0$ if a patient with a positive sputum after six weeks had an extended intensive treatment. Stage is defined as before.

Model 1 red: $logit(P = 1|treat) = \alpha_0 + \alpha_1\, treat$,

Model 2 red: $logit(P = 1|path) = \alpha_0 + \alpha_1\, path_2 + \alpha_2 path_3$

11.4 Consolidation Phase Power Simulations

11.4.1 Consolidation Power

Table 22 shows the power of the design to detect differences between oral consolidation vs oral + IA consolidation. It appears for the scenarios considered that we have good power to detect differences.

Table 21: Consolidation Power for different hypotheses, 100000 simulation replicates

Pathway	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
Oral+IA vs Oral only 1: 3+5 vs 4+6	0.051	0.86	0.63	0.83	0.95	0.90
Oral+IA vs Oral only 2: 1+3+5 vs 2+4+6	0.051	0.95	0.77	0.95	0.98	0.95

SUB-STUDIES CONDUCTED WITHIN APPENDIX A1

SUB-STUDY A1.1: SHORT INTENSIVE THERAPY

1 PRIMARY OBJECTIVE

To compare the efficacy of intensive therapies on microbiological clearance of MABS with good tolerance at 4 weeks. Specifically, the effects of inhaled versus intravenous amikacin will be examined and the efficacy of additional oral clofazimine to standard intravenous therapy will be examined.

Investigate the efficacy of intensive therapy on microbiological clearance with acceptable toxicity of treatment combinations will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies *M. a. abscessus/M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense*) and those with constitutive macrolide resistance).

Nested within sub-study A1.1 are:

A1.1.1: Use of Inhaled Amikacin (IA) During Intensive Therapy to Replace Intravenous Amikacin (IVA) in the Treatment of MABS-PD;

A1.1.2: The use of Additional Clofazimine to Standard Intravenous Therapies During Intensive Therapy in the Treatment of MABS-PD.

A1.1.1: USE OF INHALED AMIKACIN (IA) DURING INTENSIVE THERAPY TO REPLACE INTRAVENOUS AMIKACIN (IVA) IN THE TREATMENT OF MABS-PD

1 PRIMARY OBJECTIVE

To compare the microbiological clearance of MABS with good tolerability at 4 weeks of intensive therapy with the use of IA (Arm B) with the use of IV amikacin (Arm A) given during intensive phase.

2 SECONDARY OBJECTIVES

1. Microbiological clearance with acceptable toxicity at 4 weeks of intensive therapy with the use of IA (Arm B) compared with the use of IV amikacin (Arm A) during the intensive phase will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies *M. a. abscessus/M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense*) and those with constitutive macrolide resistance).
2. Microbiological clearance at week 4 (irrespective of toxicity) with use of IA (Arm B) compared with use of IVA (Arm A).
3. Microbiological clearance at week 4 (irrespective of toxicity) with use of IA (Arm B) compared with use of IVA (Arm A) in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies *M. a. abscessus/M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense*) and those with constitutive macrolide resistance).
4. Safety of using IA to replace IV amikacin in the short intensive therapy phase (6 weeks).
5. Change in FEV_1 z-score at week 6 versus Day 1 in with use of IA (Arm B) compared with use of intravenous amikacin IVA (Arm A).
6. Change in HRQoL (CFQ-R respiratory domain) for participants with CF at week 6 versus day 1 in with use of IA (Arm B) compared with use of IVA (Arm A).
7. To examine general HRQoL (SF-36 (Adults) and Peds-QL™ (Children)) between Day 1 and week 6 in participants with use of IA (Arm B) compared with use of IVA (Arm A).
8. To examine the cost effectiveness of IA compared with IVA during 6 weeks of intensive therapy.

3 INTRODUCTION

Amikacin, an aminoglycoside guideline-based therapy in the treatment of MABS-PD can be administered either intravenously or nebulized for inhalation (IV formulation). Inhaled aminoglycosides have the potential advantages of achieving higher airway concentrations, while reducing the risk of systemic toxicity. However, amikacin for inhalation is not currently available commercially for the indication of treating MABS and clinicians have used an “off-label” IV form of amikacin (delivered via a nebuliser). A Phase II RCT (30) of amikacin liposome inhalation suspension

(Arikayce) sponsored by Insmed Inc., investigated the safety and efficacy of Arikayce to treat NTM infection (36% MABS) in addition to highly variable multi-drug therapy in 89 patients with (19%) and without (81%) CF in patients who had failed to clear infection using their current treatment. The trial did not meet the primary endpoint of decreased mycobacterial load, but the treatment group had a higher proportion of subjects with >1 negative sputum (32% vs 9%, P=0.006) and improved 6- minute walk distance, suggesting a potential benefit from adding Arikayce to consolidation therapy. Arikayce has now been approved for the treatment of patients infected with *Mycobacterium avium* complex (MAC) who do not respond to conventional treatment.

4 ELIGIBILITY CRITERIA

No additional eligibility criteria are required for participation in A1.1.1 from those described in Appendix A1, Section 5.

5 ADDITIONAL SUB-STUDY SPECIFIC PROCEDURES

No sub-study specific procedures are required in addition to those described in Section 5.5, Core Trial Procedures of the Master Protocol and the procedures described in Section 10, Appendix A1. Refer to Tables 13-15 for the schedule of assessments in Appendix A1.

6 STATISTICAL ANALYSIS

Details of the simulations (sample size calculation) for this study are presented in the simulations section of Appendix A1, section 12.

The sub-study's primary microbiological endpoint of clearance of MABS at 4 weeks with good tolerance will be presented as a number and proportion in the IVA (Arm A reference group) and IA (Arm B) groups. A comparison between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value. All secondary outcomes will also be presented separately in the two treatment groups, as means and standard deviations for continuous outcomes and numbers and proportions for binary outcomes. Secondary efficacy objectives will be compared between arms using linear (continuous outcomes) and logistic (binary outcomes) regression adjusted for the stratification factors as per the primary outcome. Safety outcomes (AEs and SAEs) will be presented as a number and the proportion of participants experiencing an event and the number of events in the Arm B (IA) and Arm A (IVA). A comparison of whether or not participants experience an event between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value.

STUDY A 1.1.2: THE USE OF ADDITIONAL CLOFAZIMINE TO STANDARD INTRAVENOUS THERAPIES DURING INTENSIVE THERAPY IN THE TREATMENT OF MABS-PD

1 PRIMARY OBJECTIVES

To compare the microbiological clearance of MABS with good tolerability at 4 weeks between standard IV without clofazimine (Arm C) and with clofazimine (Arm A) given during intensive phase.

2 SECONDARY OBJECTIVES

1. Microbiological clearance with acceptable toxicity at 4 weeks of intensive therapy without (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies *M. a. abscessus/M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense*) and those with constitutive macrolide resistance).
2. Microbiological clearance (irrespective of toxicity) at week 4 without the addition of clofazimine (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment.
3. Microbiological clearance (irrespective of toxicity) at 4 weeks of intensive therapy without (Arm C) and with the addition of clofazimine (Arm A) to standard IV treatment will also be examined in different patient subpopulations (CF and non-CF, those infected with different MABS subspecies *M. a. abscessus/M. a. bolletii* [inducible macrolide resistance] and *M. a. massiliense*) and those with constitutive macrolide resistance).
4. Safety of using additional clofazimine in the short intensive therapy phase (6 weeks)
5. Change in FEV_1 z-score between Day 1 and week 6 in participants treated without additional clofazimine (Arm C) and with additional clofazimine (Arm A).
6. Changes in HRQoL (CFQ-R respiratory domain for participants with CF) between Day 1 and week 6 in participants treated without (Arm C) and treated with additional clofazimine (Arm A).
7. To examine general HRQoL (SF-36 (Adult)s and Peds-QL™ (Children)) between Day 1 and week 6 in participants treated without (Arm C) with additional clofazimine (Arm A).
8. To examine the cost effectiveness of additional clofazimine during 6 weeks of intensive therapy in addition to standard IV treatment.

3 INTRODUCTION

Clofazimine is approved for use in leprosy. It has recognised antibacterial and anti-inflammatory effects in the management of leprosy and has been used to treat erythema nodosum related to leprosy. There is no current clinical trial evidence to support the use of clofazimine in the treatment of MABS-PD. Clofazimine use has however increased clinically potentially driven by the difficulty in achieving

microbiological clearance and reports of *in vitro* synergy between combinations of clofazimine and amikacin (61, 62).

4 ELIGIBILITY CRITERIA

No additional eligibility criteria are required for participation in sub-study A1.1.2 from those described in Appendix A1, section 5.

5 ADDITIONAL SUB-STUDY SPECIFIC PROCEDURES

No sub-study specific procedures are required in addition to the Core Trial Procedures (section 5.6 of the Master Protocol) and the procedures outlined in section 10 of Appendix A1.

6 STATISTICAL ANALYSIS

Details of the sample size calculation for this study are presented in statistical analysis the simulations section of Appendix A1, section 12.

The sub-study's primary microbiological endpoint of clearance of MABS at 4 weeks with good tolerance will be presented as a number and proportion in the group with additional clofazimine (Arm A reference group) and the group with no additional clofazimine (Arm C). A comparison between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value. All secondary outcomes will also be presented separately in the two treatment groups, as means and standard deviations for continuous outcomes and numbers and proportions for binary outcomes. Secondary efficacy objectives will be compared between arms using linear (continuous outcomes) and logistic (binary outcomes) regression adjusted for the stratification factors as per the primary outcome. Safety outcomes (AEs and SAEs) will be presented as a number and proportion of participants experiencing an event and the number of events in the clofazimine (Arm A) and non-clofazimine groups (Arm C). A comparison of whether or not participants experience an event between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value.

SUB-STUDY A 1.2: DURATION OF INTENSIVE THERAPY FOR PATIENTS WITH ONGOING POSITIVE MABS CULTURES AFTER 4 WEEKS OF INTENSIVE THERAPY.

1 PRIMARY OBJECTIVE

To compare the microbiological clearance with good tolerability at 10 weeks in patients who have ongoing MABS positive cultures at week 4, between those who are allocated to completion of prolonged (12 weeks) of intensive therapy and those who are allocated to consolidation following a shorter (6 weeks) intensive therapy.

2 SECONDARY OBJECTIVES

1- To compare microbiological clearance at 10 weeks (irrespective of toxicity) in prolonged intensive compared with short intensive + consolidation in patients who had MABS positive cultures at 4 weeks.
2- Safety of prolonged intensive therapy phase compared with short intensive therapy and consolidation (12 weeks).
3- Change in FEV_1 z-score between Day 1 and week 12 in those who receive prolonged intensive compared with short intensive + consolidation in patients with MABS positive cultures for MABS at 4 weeks.
4- Change in FEV1 z-score at week 12 between those participants still culture positive for MABS at 10 weeks compared with those who have cleared MABS at 10 weeks.
5- Changes in HRQoL (CFQ-R for CF) in prolonged intensive compared with short intensive + consolidation in patients who had MABS positive cultures at 4 weeks.
6- Change in CT scan parameters (bronchiectasis, mucus plugging, airway wall thickening, -- atelectasis, % disease and air trapping), between screening and 12 weeks taking into account microbiological clearance at 4 and at 10 weeks.
7- To examine the cost effectiveness of intensive therapy prolonged to 12 weeks compared with 6 weeks of intensive therapy + 6 weeks consolidation for those who remain MABS positive at 4 weeks of intensive therapy.

3 INTRODUCTION

Current published guidelines for the treatment of MABS-PD (1, 4, 54) are based on expert opinion, and in practice treatments vary considerably (29). Suggested regimens include an intensive phase of 4-12 weeks of IV antibiotics with duration based on clinical and microbiological response. Whether extending the length of intensive therapy improves microbiological clearance of MABS is not known but the strategy is used clinically in patients with ongoing positive cultures. Toxicity and costs are

thought to be related to length of treatment and need to be balanced against the potential for better microbiological outcomes.

4 ELIGIBILITY CRITERIA

Participants are required to have completed 6 weeks of intensive therapy and will have at least one out of 3 sputum cultures or one BAL culture positive for MABS collected at week 4 (± 3 days).

No other additional eligibility criteria are required for participation in sub-study A1.2 from those described in Section 5 of Appendix A1.

5 ADDITIONAL SUB-STUDY SPECIFIC PROCEDURES

No sub-study specific procedures are required in addition to the Core Trial Procedures (section 5.6 of the Master Protocol) and the procedures outlined in section 10 of Appendix A1.

6 STATISTICAL ANALYSIS

Details of the sample size calculation for this study are presented in the simulations section in the Appendix A1, section 12.

The sub-study's primary microbiological endpoint of clearance of MABS at 10 weeks with good tolerance will be presented as a number and proportion in the group with positive cultures at 4 weeks allocated either to prolonged intensive or short intensive and consolidation. A comparison between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value. All secondary outcomes will also be presented separately in the two treatment groups, as means and standard deviations for continuous outcomes and numbers and proportions for binary outcomes. Secondary efficacy objectives will be compared between arms using linear (continuous outcomes) and logistic (binary outcomes) regression adjusted for the stratification factors as per the primary outcome. Safety outcomes (AEs and SAEs) will be presented as a number and proportion of participants experiencing an event and the number of events in the prolonged intensive and short intensive + consolidation groups. A comparison between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value.

SUB-STUDY A 1.3: CONSOLIDATION THERAPY

1 PRIMARY OBJECTIVE

To compare the microbiological clearance with good tolerability of MABS between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA at either 56 or 62 weeks depending on clearance of MABS at 4 and 10 weeks.

2 SECONDARY OBJECTIVES

1. To compare microbiological clearance (irrespective of toxicity) at completion of consolidation therapy between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy with additional IA at either week 56 or 62 depending on clearance of MABS at week 4 and 10 weeks.
2. Safety of oral only versus oral and inhaled therapy for consolidation treatment of MABS.
3. Change in FEV_1 z-score between start and end of consolidation therapy (weeks 6 and 56 or weeks 12 and 62) between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA taking into account microbiological clearance at 4 and at 10 weeks.
4. Change in CT scan parameters (bronchiectasis, mucus plugging, airway wall thickening, atelectasis, % disease and air trapping), between 12 weeks and at end of consolidation either 56 or 62 weeks taking into account microbiological clearance at 4 and at 10 weeks between those between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
5. Changes in HRQoL (CFQ-R for CF) between start and end of consolidation in those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and additional IA.
6. To examine general HRQoL (SF-36 (Adults) and Peds-QL™ (Children)) between start and end of consolidation in adults (CF and non-CF) (weeks 6 and 56 or weeks 12 and 62) between those allocated to consolidation therapy with oral treatment and those allocated to consolidation therapy with oral therapy and those allocated to oral therapy with additional IA.
7. To compare the cost effectiveness of consolidation between those allocated to consolidation therapy with oral therapy and those allocated to oral therapy with additional IA.

3 INTRODUCTION

Currently, the guidelines for consolidation (54) suggest that patients with isolates that are sensitive to macrolides or that have inducible resistance should be managed with a combination of between one and three oral antibiotics based on drug susceptibility of the isolate and tolerance in combination with IA. Those with isolates that have constitutive resistance should use a combination of between two and four

oral drugs based on susceptibility and tolerance in combination with IA. In addition, guidelines suggest that patients with isolates that have amikacin resistance could substitute IA for an alternative oral antibiotic. It is suggested that treatment should continue for 12 months after culture conversion.

There is currently no evidence for any of these guidelines. IA is costly and time and effort for maintenance of hygienic practice and drug delivery. Furthermore, adherence to inhaled therapies over the longer term is variable (63-65). This study will examine the effects of IA in addition to the oral only thus providing some evidence regarding the use of additional IA during consolidation. The timing of consolidation therapy is only 46 weeks for this trial and patients are free to continue treatments after completion of the trial. Microbiological clearance will be determined from culture of respiratory samples (sputum (expectorated or induced) or BAL) collected ***at least one week apart*** as follows:

1. Participants who continue to produce sputum samples during the consolidation phase are requested to provide additional sputum samples on three additional occasions towards the end of consolidation therapy (Weeks 52 for the short intensive group or Week 58 for the prolonged intensive group). These three sputum samples collected towards the end of treatment (or after the end of consolidation but prior to the final outcome) are to be collected at least one week apart and will determine the consolidation therapy microbiological clearance. A final sputum sample collected four weeks after cessation of consolidation therapy will determine the final outcome microbiological clearance.
    a. For participants who continue to have positive sputum cultures at week 52 or week 58, a gap in therapy to complete final outcomes at week 56 or week 62 is not required as microbiological clearance will not have occurred.
    b. Participants who have negative cultures on treatment at end of consolidation will need to cease treatment for 4 weeks for final outcome to enable assessment of microbiological clearance without antibiotic suppression.
2. For participants who are unproductive of sputum during the consolidation phase, or who become unproductive of sputum during the course of consolidation therapy, a BAL is to be performed at final outcome (Weeks 56 for short intensive or 62 for long intensive), the results of which will also inform the consolidation therapy outcome.

4 ELIGIBILITY CRITERIA

Participants who have completed 6 weeks of intensive therapy are eligible for participation in A1.3. No additional eligibility criteria are required for participation in sub-study A1.3 from those described in Section 5, Appendix A1.

5 ADDITIONAL SUB-STUDY SPECIFIC PROCEDURES

No sub-study specific procedures are required in addition to the Core Trial Procedures (section 5.6 of the Master Protocol) and the procedures outlined in section 10 of Appendix A1.

6 STATISTICAL ANALYSIS

Details of the sample size calculation for this study are presented in the simulations section, Appendix A1, section 12.

The sub-study's primary microbiological endpoint of clearance of MABS with good tolerance at 52 or 58 weeks will be presented as a number and proportion separately for those allocated to consolidation therapy with oral therapy only and those with additional IA. For participants who are unproductive during the consolidation phase between Week 18 and Weeks 52 or 58 or have not provided enough sputum samples during this time period, a single BAL sample collected at Week 56 or 62 will be used as the consolidation outcome. A comparison between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation, as well as clearance at 4 weeks and 10 weeks. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value. All secondary outcomes will also be presented separately in the two treatment groups, as means and standard deviations for continuous outcomes, and numbers and proportions for binary outcomes. Secondary efficacy objectives will be compared between arms using linear (continuous outcomes) and logistic (binary outcomes) regression adjusted for the stratification factors as per the primary outcome as well as clearance at 4 weeks and 10 weeks. Safety outcomes (AEs and SAEs) will be presented as a number and proportion of participants experiencing an event and the number of events in the prolonged intensive and short intensive + consolidation groups. A comparison between the groups will be conducted using logistic regression adjusted for the stratification factors as used in randomisation. Results will be presented as an (adjusted) odds ratio, along with its 95% confidence interval and p-value.

7 APPLICABLE DISCOVERY STUDIES FOR APPENDIX A1

1. B1.1: Steady state pharmacokinetics of amikacin.
2. B1.2: Micro sampling and non-blood matrix validation for amikacin therapeutic drug monitoring.
3. B1.3: Pharmacokinetics of MABS-PD therapies.
4. B2.1: Macrophage function.
5. B2.2: Mitochondrial stress.
6. B2.3: Gene expression signatures.
7. B2.3: T cell function.
8. B2.4: Serology for MABS.
9. B3.1: Imaging.
10. Appendix C: Australian CF Data Registry.

APPENDIX B: DISCOVERY

Appendix B: Discovery, consist of research studies/programs that may be added over time and that are aimed at gaining understanding through collaboration with the FOR*Ma*T trial but are not specific components of the trial. Participation in each discovery study may require additional consent. Consent requirements are detailed within each of the discovery sub-studies. Discovery studies may be applicable to all participants or only to specific sites or groups of participants. Each study will be described in the following format:

- The named Investigator leads for the sub-study;
- The primary and secondary objectives of the sub-study;
- An introduction to the background of the sub-study;
- Additional eligibility and consent requirements;
- Sub-study specific trial procedures and schedule of assessments;
- Analysis plan.

Appendix B1: Pharmacokinetics.

Appendix B2: Immune Factors and Biomarkers.

Appendix B3: Imaging.

Appendix B4: Vestibular.

APPENDIX B1: PHARMACOKINETICS

Investigator Leads

Dr Andrew Burke and Professor Jason Roberts

Introduction

Appendix B1, Pharmacokinetics addresses a key research question posed by the FOR*Ma*T trial relating to the optimal use of drug therapies in MABS infection. Answering this question has been hampered by the lack of pharmacokinetic (PK) studies in people with mycobacterial lung disease. Nested within Appendix B1 are three PK sub-studies:

SUB-STUDY B1.1: Steady State Pharmacokinetics of Amikacin;

SUB-STUDY B1.2: Micro Sampling and Non-Blood Matrix Validation for Amikacin Therapeutic Drug Monitoring;

SUB-STUDY B1.3: Pharmacokinetics of MABS-PD Therapies.

SUB-STUDY B1.1: STEADY STATE PHARMACOKINETICS OF AMIKACIN

1 PRIMARY OBJECTIVE

Determine the best practice therapeutic drug monitoring recommendations for use of intravenous amikacin to maximise antimicrobial effect and minimise toxicity in the treatment of MABS-PD.

2 INTRODUCTION

The optimal use of amikacin in the treatment of MABS-PD is hampered by the lack of PK studies in in all people with mycobacterial lung disease (66). There are no current validated Cmax or AUC targets for amikacin use in the management of NTM disease, with or without concomitant MIC data. The development of novel anti-mycobacterial drug assays at the University of Queensland Centre for Clinical Research (UQCCR) and Pathology Queensland will allow accurate measurement of drug concentrations in plasma and other body fluids including BAL, sputum and oral fluids. This data can then be analysed to inform our understanding of the PK of amikacin in MABS-PD patients. It will also allow for the rational design of future studies on TDM for amikacin which has a narrow therapeutic window. The increased use of TDM has been suggested in the treatment of multidrug resistant TB (MDR-TB) as well as for complicated NTM infections. A better understanding of the PK of amikacin will therefore allow us to optimise dosing to minimise toxicity while maximising the likelihood of cure.

3 ADDITIONAL ELIGIBILITY CRITERIA

In addition to meeting the eligibility criteria described in the Master Protocol, participants are required to meet the following eligibility criteria:

3.1 Additional Inclusion Criteria

- Participant is allocated to a treatment arm receiving IV amikacin therapy,
- Availability of suitable venous access to facilitate sample collection,
- Availability of patient demographics including weight and height,
- Accurate recording of time of drug administration and infusion time,
- Informed consent by participant or participants parent/guardian.

3.2 Additional Exclusion Criteria

- Unavailability of venous access for blood collection,
- No record of timing or duration of infusion,
- Participants receiving nebulised instead of intravenous amikacin,
- Participants enrolled in the observation program.

4 ADDITIONAL INFORMED CONSENT

As amikacin TDM is already standard of care and amikacin levels are already being collected sub-study specific patient consent will not be required as no additional collections will be undertaken.

5 AMIKACIN TDM PROCEDURES

There is currently variation in practice surrounding amikacin dosing and TDM and this is likely to be reflected in the recruiting centres for the FOR*Ma*T trial. Some patients will be receiving intermittent (second or third daily dosing) and others once daily dosing. The current TDM strategies that can be used in the FOR*Ma*T trial include;

- Trough amikacin levels (independent of MIC) using nomogram for next dose,
- Cmax/MIC target,
- AUC target,
- AUC/MIC target.

As TDM for amikacin is already standard of care and is incorporated into the trial Master Protocol, all sampling events will be recorded in the FOR*Ma*T trial REDCap database. Investigators will be free to use the dosing and TDM method that is their usual practice, however it will be documented what approach is being used to allow comparison between different strategies.

Participants will have blood drawn through a suitable venous access device and the sample will be processed at local laboratory as per usual practice for that trial site. Results will be recorded as will time, dose of amikacin and frequency of dosing. Same day serum creatinine concentrations as well as admission weight and height will be recorded.

All amikacin levels accompanied by a serum creatinine concentration within 24 hours of sampling will be included in analysis. Relevant demographic data e.g. weight and height, precise time that study drugs have been administered and method of TDM utilised by treating clinicians will be recorded in the CRF.

6 PHARMACOKINETIC ANALYSIS

To describe amikacin concentrations, one and two-compartment models will be evaluated using the Nonparametric Adaptive Grid (NPAG) algorithm within the Pmetrics software package for R (Los Angeles, CA). Elimination from the central compartment, and intercompartmental distribution into the peripheral compartment (two compartment model) will be modelled as first-order processes. Discrimination between different models used comparison of the -2 log likelihood (-2LL). A p-value of <0.05 was considered statistically significant.

Age, sex, body weight, renal function descriptors and other clinical descriptors will be tested as covariates. Covariate selection will be performed using a stepwise linear regression from R on all covariates and Bayesian posterior parameters. Potential covariates will be separately entered into the

model and statistically tested by use of the -2LL values. If inclusion of the covariate results in a statistically significant improvement in the -2LL values ($p<0.05$) and/or improved the goodness-of-fit plots, then it will be retained in the final model.

6.1 Model Diagnostics

Goodness-of-fit will be assessed by linear regression, with an observed-predicted plot, coefficients of determination, and log-likelihood values. Predictive performance evaluation will be based on mean prediction error (bias) and the mean bias-adjusted squared prediction error (imprecision) of the population and individual prediction models. The internal validity of the population pharmacokinetic model will be assessed by the bootstrap resampling method (n=1000) and normalized prediction distribution errors (NPDEs). Using visual predictive check (VPC) method, parameters obtained from the bootstrap method will be plotted with the observed concentrations.

Once the pharmacokinetic model has been developed, amikacin exposure in enrolled patients will be simulated as area under the concentration-time curve (AUC) from 0-24 hours and peak concentration (Cmax) during a dosing interval. The association of clinical cure versus amikacin exposure will be tested using a Mann-Whitney U-test. A classification and regression tree (CART) analysis will be used to propose breakpoints (e.g. Cmax, Cmax/MIC, AUC, AUC/MIC) which quantify the exposures associated with maximal effectiveness and can be used to guide future TDM processes.

SUB-STUDY B1.2: MICRO SAMPLING AND NON-BLOOD MATRIX VALIDATION FOR AMIKACIN THERAPEUTIC DRUG MONITORING

1 PRIMARY OBJECTIVE

Compare less intrusive micro sampling strategies to the gold standard methods for amikacin TDM.

2 INTRODUCTION

Patients are on treatment for MABS-PD for months and repeated blood sampling for TDM and PK analysis currently requires venepuncture and on-site centrifuge prior to transport offsite for testing. If same day testing cannot be done, then the sample requires freezing. These factors cause discomfort to the patient and impose logistical barriers to PK studies in clinical trials as well as limiting the availability of TDM and its ability to influence prescribing decisions through real time feedback to clinicians.

Less intrusive sampling strategies have been proposed, however, it is unknown whether these strategies can accurately measure amikacin levels in a diverse range of body fluids. Dried blood spot (DBS) and Volumetric Absorbent Micro sampling (VAMS) performed through finger prick testing (Appendix D) may be a practical surrogate for traditional methods of obtaining plasma from venepuncture. Other sampling technologies that will be trialled include: hemaPEN, safeClinitube and Drummond plasma gel separator tubes.

As NTM infection is situated in the lower respiratory tract the concentration of drugs in the respiratory epithelial fluid is the PK endpoint of most relevance. Sampling this compartment has traditionally required bronchoscopy with BAL. As well as being an invasive procedure requiring sedation, lung inflammation and mucous impaction as found in CF may contribute to sampling and dilutional error. Suitable alternatives to be investigated include sputum and oral fluid to specimens obtained from BAL. Simpler and more reliable sampling methods testing oral fluid drug concentrations on salivette cotton swabs are already an established and well tolerated method for obtaining cortisol levels in clinical practice (Appendix D) but further investigation into the utility of these samples in MABS-PD is required.

3 ADDITIONAL ELIGIBILITY CRITERIA

Participants at nominated Australian FOR*Ma*T trial sites will be eligible to participate in sub-study B1.2. In addition, participants eligibility will be based on the following criteria:

3.1 Inclusion criteria

- Receiving intravenous amikacin as part of FOR*Ma*T intervention program,
- Availability of suitable intravenous access to facilitate sample collection,
- Patients may be receiving other antibiotics concurrently,

- Informed consent by participant or participants parent/guardian.

3.2 Exclusion criteria

- Unavailability of suitable venous access device for blood collection after consultation with the treating team,
- Enrolled in the observation program.

4 ADDITIONAL INFORMED CONSENT

Additional blood samples are required as part of the study procedures for sub-study B1.2, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

5 MICRO SAMPLING PROCEDURES

The day of testing will be determined by the site Investigator and will be after **5 half-lives** of IV amikacin i.e. at least 48 hours to ensure testing is done at steady state.

1. Participants receiving IV amikacin will have samples taken using two micro sampling techniques (detailed below) in addition to whole blood collection through phlebotomy.
2. In addition to the amikacin trough and peak levels collected for standard of care, samples will be collected at three time points; 0 hours (time amikacin infusion ends), 0.5 hours and 1 hour post amikacin infusion.
3. At each time point the following blood samples will be collected from the participant: 1 venous whole blood sample (4ml EDTA tube) and a sample from two micro sampling devices.
4. Micro sampling devises to be tested include: VAMS, hemaPEN, safeClinitube Drummond plasma gel separator tubes and DBS.
5. Whole blood will be drawn through a suitable venous access device and the sample will be processed at local laboratory as per usual practice for that trial site.
6. Same day haematocrit, albumin and creatinine testing will be done up to 5mls of blood collected by venous sampling in an EDTA tube. Creatinine and albumin can be measured from the heparinised sample which is already being collected for amikacin levels.
7. At the same time as a whole blood sample is being collected a salivette swab will be inserted into the mouth. It will be removed after being chewed for 2 minutes and will be processed as per appendix D.
8. A brief patient or care giver questionnaire will be undertaken to determine level of discomfort with micro sampling techniques compared to venepuncture as well as degree of technique acceptability to research nurse.
9. Amikacin assay results and other relevant pathology results will be recorded as will time, dose and frequency of dosing of each IV drug in the sub-study specific CRF.

At each time point the following blood samples will be collected from the patient: 1 venous whole blood sample (4 ml EDTA tube) and a sample from two micro sampling devices with either;

- 1 finger prick sample using 1 Guthrie card for dried blood spot (DBS) ~ 50 µl of blood, OR;
- a finger prick sample using 2 Mitra® VAMS (a fraction of a bead of blood (10µL) x2), OR;
- a finger prick sample using haemaPEN (a fraction of a bead of blood (2.5µL) x4), OR;
- safeClinitube (50 µL equivalent to a drop of blood), OR;
- Drummond plasma gel separator tubes (50 µL equivalent to a drop of blood).

5.1 Choice of Micro Sampling Device

The choice of which micro sampling device will be used will be determined by the lead Investigator for the PK sub-studies. To maintain consistency in technique and to ensure recruitment of sufficient numbers in each testing arm, centres will be allocated a micro sampling device to test.

Once participants have consented to sub-study B1.2 this information will be entered into the FOR*Ma*T REDCap database. The FOR*Ma*T site research team will be contacted by the FOR*Ma*T Management Team notifying them of the micro sampling device allocated to that participant. This information, along with the TDM strategy employed will also be recorded in the participants database record.

Once 25 separate paired amikacin samples have been collected further testing on this device will cease pending results of analysis. 15-20 subjects will be required per device depending on the TDM strategy used. Once a study site has completed target enrolment in the allocated microsampling device they will be allocated another device to trial. Devices will be allocated in the order of: VAMS, DBS, haemaPEN, safeClinitube and Drummond plasma gel separator tubes. Samples will be collected as per methodology described in Appendix D.

5.2 Opportunistic BAL Sampling

For those participants having bronchoscopy as part of their normal clinical care, BAL samples will be transported to UQCCR on the next working day for drug concentration testing of drugs that they are already on as part of their normal care (see Appendix D). To determine whether saliva can be used as a surrogate for BAL concentrations, a salivette swab will be inserted into the mouth before bronchoscopy and drug assays performed for study drugs including amikacin. Bronchoscopy will only be performed based on clinical need and not for the primary purpose of PK studies.

6 STATISTICAL ANALYSIS PLAN

The aim of the microsampling study will be to assess the utility and validate different microsampling devices. A formal PK analysis will not be performed on these specimens as this will have already been conducted on plasma samples. Validation of microsampling techniques will be performed with reference to the FDA document on bioanalysis. Intra and inter-assay precision and accuracy will be

determined for each technique as will the lower limit of quantification (LLOQ), linearity, matrix effects, recovery, selectivity and stability of dried matrix.

SUB-STUDY B1.3: PHARMACOKINETICS OF MABS-PD THERAPIES

1 PRIMARY OBJECTIVE

Assess steady state pharmacokinetics in participants enrolled in the Intervention Program for the treatment of MABS-PD.

2 INTRODUCTION

Population pharmacokinetic modelling of samples across one dosing interval can give more reliable PK information than traditional PK analysis. This allows for a more efficient and streamlined study on a smaller number of patients. Given the increasing treatment burden for MABS-PD a more comprehensive understanding of the PK of these drugs is imperative in the development of safer and more effective therapies for patients with MABS-PD. There are no data currently available relating to pharmacokinetics of drugs commonly used in the management of MABS-PD.

3 ADDITIONAL ELIGIBILITY CRITERIA

In addition to meeting the eligibility criteria described in the FOR*Ma*T Master Protocol, participants are required to meet the eligibility criteria outlined below to enrol in sub-study B1.3:

3.1 Inclusion criteria

- Enrolled in the intervention program at an Australian site,
- Availability of suitable intravenous access to facilitate sample collection,
- Patients may be receiving other antibiotics concurrently,
- Informed consent by participant or participants parent/guardian.

3.2 Exclusion criteria

- Unavailability of suitable intravenous access device for blood collection,
- Treating clinician advice,
- Enrolled in the observation program.

4 ADDITIONAL INFORMED CONSENT

Additional blood samples are required as part of the study procedures for sub-study B1.3, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

5 SUB-STUDY SPECIFIC PROCEDURES

1. The day of study will be determined by the site Investigators and will be after **5 half-lives** of the drug with the longest half-life (excluding azithromycin which has a prolonged half-life) to ensure testing is done at steady state.

2. Participants will have up to 5 ml whole blood through a cannula at the following times on a single day of intensive therapy treatment:
    i. immediately pre-dose (trough level),
    ii. post- dosing: 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, and 8 hours,
    iii. Children may have opportunistic testing of blood concentrations based on samples that are already being collected as part of routine care (i.e. "salvaged" bloods).
3. The exact time of each blood draw will be recorded in the sub-study CRF. If the participant is on multiple IV antibiotics, the commencement of the infusion of each IV antibiotic will occur at different times. The timings for commencement and completion of each IV drug will therefore be recorded on the data entry sheet.
4. On the same day, a salivette swab will be inserted into the mouth at each time point blood is being collected. The salivette swab will be removed after being chewed for 2 minutes and will be transported to Queensland Pathology, Herston Brisbane the next working day in accordance with procedures detailed in Appendix D.

6 STATISTICAL ANALYSIS

Enrolment targets for sub-study B1.3 are; 50 participants ≥18 years of age and 30 participants subjects <18 years of age. Participants will be on different antibiotic therapies, therefore this allocation of participants will allow for a minimum of 20 participants per antibiotic.

PK indices will include volumes of distribution, C_{max}, AUC and clearance. A pharmacokinetic model will also be developed using a population pharmacokinetic approach. This technique will enable us to quantify the effect of patient and treatment characteristics on altered pharmacokinetics (e.g. patient age, weight, renal function, hepatic function, interacting drugs). This model can then be used to assess the adequacy of current dosing regimens and can also be used for dosing simulations to define optimised dosing regimens for these patients.

PK analysis will be conducted with Pmetrics software package for R (version 1.4.1., Los Angeles, CA). Non-linear mixed effect modelling approach will be used. A stepwise analysis will be performed to first determine the structural base model by fitting the concentration-time data to one, two and three compartment models. Testing of additive, proportional or a combination of additive and proportional models will be conducted to select the best fit statistical error model. Following population PK analysis, Monte Carlo simulations will be performed using MICs for target NTM pathogens derived from clinical specimens tested at Pathology Queensland's Mycobacterial Reference laboratory (MRL). These PK/PD parameters can be used to estimate appropriate doses in clinical practice.

APPENDIX B2: IMMUNE FACTORS AND BIOMARKERS

Introduction

Appendix B2 sub-studies will take advantage of careful clinical and microbiological phenotyping of participants enrolled in the FOR*Ma*T Master Protocol to examine immunological susceptibility to infection with MABS-PD providing an opportunity to develop new approaches to assessing risk of infection as well as approaches to management in the future. A summary of sample requirements and volumes are outlined in Table 22.

SUB-STUDY B2.1: Macrophage Function.

SUB-STUDY B2.2: Mitochondrial Stress.

SUB-STUDY B2.3: Gene Expression Signatures.

SUB-STUDY B2.4: T-Cell Function.

SUB-STUDY B2.5: Serology for MABS.

SUB-STUDY B2.1: MACROPHAGE FUNCTION

Investigator Leads

Professor Peter Sly and Dr Abdullah Tarique

1 PRIMARY OBJECTIVE

Determine susceptibility to MABS infection in participants enrolled in the observational program compared to those with MABS-PD enrolled in the intervention program by examining impaired function of MABS infected macrophages.

2 SECONDARY OBJECTIVES

1. Determine efficiency of macrophage phagocytosis and killing of MABS.
2. Determine whether decreased efficiency of macrophage phagocytosis and killing of MABS contributes to the likelihood of chronic infection with MABS-PD.
3. Determine whether inflammatory signalling pathways activated following MABS-PD infection are dysregulated in macrophages.
4. Examine the association between aberrant phagocytosis and enhanced mitochondrial reactive oxygen species (ROS) production in macrophages in participants following MABS infection.

3 INTRODUCTION

MABS exists in two morphotypes, rough (R) and smooth (S), depending on the presence of surface glycopeptidolipids (GPLs) (67). Rough MABS (MABS-R), which lack surface GLP expression, and smooth MABS are both phagocytized by human monocytes and macrophages. MABS-R have greater ability for intracellular multiplication and adhere more tightly to the phagosomal membrane (68). Macrophages play critical roles in the initiation and resolution of pulmonary inflammation. Following microbial infection, macrophages recognise the invading bacteria via specific surface receptors including Toll-like receptors (TLRs) to engulf and phagocytose them into phagosomes, a compartment that restricts the mobility of the invading bacteria. Phagosomes then fuse with acidic lysosomal compartments to initiate bacterial killing. TLR signalling initiates inflammatory responses to the pathogen. MABS-S are recognised via both Dectin-1, a C-type lectin molecule and TLR2 (69, 70). A physical interaction between Dectin-1 and TLR2 is required to initiate phagocytosis. FAM19A4, a recently described chemokine released by macrophages following bacterial infection, is involved in phagocytosis regulation in macrophages (71).

CF macrophages have a reduced ability to phagocytose and kill bacteria. Murine studies showed that functional CFTR is indispensable for phagolysomal killing of bacteria (72). Lysosomes from CFTR knockout mice were less acidic than from wild-type mice, which led to reduced bacterial killing.

Inhibiting CFTR channel activity with specific inhibitors during phagocytosis significantly reduced lysosomal acidification, similar to reduced bacterial killing by human macrophages (73). Furthermore, both murine and human studies showed CFTR protein as a regulator of inflammatory responses to bacterial infection (74, 75). Loss of CFTR protein or function led to uncontrolled inflammation in CF. Macrophages from healthy individuals often display "don't eat me" signals by surface expression of CD31 and CD47 molecules (76, 77). Expression of TLR2, Dectin-1, regulators of phagocytosis and "don't eat me" molecules in macrophages from patients with CF have not been adequately studied.

4 ADDITIONAL ELIGIBILITY CRITERIA

Only participants enrolled at Queensland FOR*Ma*T trial sites will be eligible to participate in sub-study B2.1. In addition, participants eligibility will be based on the following criteria:

4.1 Inclusion Criteria

- Participant is enrolled at the following sites:
    - Queensland Children's Hospital,
    - The Prince Charles Hospital,
    - Princess Alexandra Hospital,
    - Royal Brisbane and Women's Hospital,
    - Gold Coast University Hospital,
    - Greenslopes Private Hospital,
    - Mater Adults Hospital,
    - Sunshine Coast University Hospital.
- Aged six years and older.
- Ability to provide a minimum 5ml whole blood sample.
- Enrolled in either the observation or intervention programs.
- Participant is enrolled at a FOR*Ma*T Trial site that can comply with sampling requirements.
- Informed consent by participant or participants parent/guardian.

4.2 Exclusion Criteria

No additional exclusionary criteria to those outlined in the FOR*Ma*T Master Protocol apply to sub-study B2.1.

5 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study B2.1, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

6 SUB-STUDY SPECIFIC PROCEDURES

5ml of whole blood is collected in participants 6-11 years of age. Participants 12 years and older will be asked to provide a 10ml whole blood sample. In participants 12 years and older unable to provide a 10ml whole blood sample, a 5ml whole blood sample is acceptable. Whole blood samples will be collected by a phlebotomist into a EDTA collection tube and transported to Centre Children's Health Research (CCHR), South Brisbane **within two hours of collection**. Trial identification (ID) number, date and time of collection are to be recorded on the sample tube and sub-study CRF.

Samples will be collected in accordance with Table 23: Sub-study B2.1 Schedule of Assessments. The collection, transport and storage of samples is in accordance with the macrophage and mitochondrial SOP's described in Appendix D.

Observational cohort participants are required to provided samples at either week 52 and 56 or week 58 and 62, not both.

Table 22: Sub-study B2.1 Schedule of Assessments

ASSESSMENT	STUDY VISITS					
	Screening Visit (Day 0)	**Week 6**	**Week 12**	**Week 28**	**End of consolidation***	**Final Outcome^**
Observation Cohort Participant Timeframes	-14 days	+14 Days	±14 Days	±14 Days	NA	±14 Days
Intervention Program Participant Timeframes	-14 Days	±3 Days	±3 Days	±5 Days	+5 Days	+14 Days
Review Eligibility for Sub-Study B2.1	✓					
Macrophage Sample	✓	✓	✓	✓	✓	✓

*Intervention Program participants only. For Intervention Program participants randomised to Week short intensive, this is collected at Week 52. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 58.
^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.

7 STATISTICAL ANALYSIS

Based on previous experience, the data generated in these experiments are likely to be non-normally distributed (using Shapiro-Wilk test), in which case grouped data will be described by group median (27^{th} and 75^{th} percentile). Comparisons between groups will be undertaken using Mann-Whitney U-Test (Wilcoxon rank sum test), Kruskal-Wallis test on ranks (non-parametric alternative to one-way

analysis of variance), or Friedman test (non-parametric alternative to analysis of variance with repeated measures), as appropriate. Regression analyses will be used to control for likely confounder or modifier variables, including age, sex, previous treatment, current CFT corrector/potentiators therapy. The techniques used for regression analyses will depend on the final sample size. If large enough, parametric methods will be used as the key assumption is that the residuals of the regression are normally distributed, and the Central Limit Theorem tells us that as the sample size increases all distributions of residuals tend towards normality. If the sample size is not sufficient a multivariable model using quantile regression, in which the median difference and 95% CI between groups can be determined.

SUB-STUDY B2.2: MITOCHONDRIAL STRESS

Investigator Leads

Professor Peter Sly and Dr Abdullah Tarique

1 PRIMARY OBJECTIVE

Determine susceptibility to MABS infection in participants enrolled in the observational program compared to those with MABS-PD enrolled in the intervention program by examining mitochondrial function.

2 SECONDARY OBJECTIVES

Examine the association between aberrant phagocytosis and reduced mitochondrial function in macrophages in participants following MABS infection.

3 INTRODUCTION

Macrophages recognize the invading bacteria via specific surface receptors to engulf and phagocytose them into phagosomes, a compartment that restrict the mobility of the invading bacteria. Phagosomes then fuse with acidic lysosomal compartments to initiate bacterial killing. Concurrent with this, specific bacterial products activate pattern recognition receptors such as the Toll-like receptors (TLRs) and C-type Lectin Receptors (CLRs) to induce both antimicrobial and inflammatory responses to the pathogen. MABSs are recognized by the CLR, Dectin-1, as well as TLR2 (69, 70). A physical interaction between Dectin-1 and TLR2 is required to initiate phagocytosis. We have described CFTR-dependent dysfunction in MDMs in CF that result in a decreased ability of M1s to kill phagocytosed bacteria and to polarize into the M2 phenotype (73). These defects are related to a post-transcriptional defect of surface expression of the IL-13Rα, required for polarization into M2s. This defect is CFTR-dependent, as it is also seen when CFTR function is inhibited in MDMs from healthy controls. In macrophages, CFTR functions as a charge shunt lowering the pH of lysosomes sufficiently to enable bacterial killing. However, CFTR function is also required for generation of ROS, an important component of intracellular bacterial killing (78). Thus, macrophages with deficient CFTR function are likely to have limited ability to kill and clear MABS.

Macrophages use a variety of methods for bacterial killing, including phagocytosis-coupled ROS generation and lysosome-mediated degradation. As noted above, CFTR function is important for these rapid antimicrobial responses. In addition, TLR signalling induces a suite of late-stage antimicrobial responses that facilitate clearance of persistent intracellular bacteria (79). These include redistribution of intracellular zinc for metal ion poisoning of bacteria (80) as well as mitochondrial reprogramming to generate antimicrobial products such as itaconic acid (81)and mitoROS (82). Mitochondria are dynamic organelles that, depending on the cellular environment, can exist as a complex network (driven by

mitochondrial fusion) or as fragmented organelles (driven by mitochondrial fission, hereafter fission). TLR signalling induces fission in macrophages (83), and we recently identified fission as a novel antimicrobial response in macrophages (unpublished). We also found that professional intra-macrophage pathogens subvert fission, and we have devised strategies to overcome this subversion to enable macrophage-mediated bacterial clearance (unpublished). Specifically, deacetylation of mitochondrial fusion-promoting mitofusin-1 by histone deacetylase (HDAC) 6 constrains fission (84). Thus, inhibiting HDAC6 with a specific inhibitor (tubastatin A) greatly enhances fission and mitoROS generation in bacterially-infected macrophages. However, the roles of antimicrobial mechanisms such as mitoROS and fission in MDM responses against MABS, and whether these responses are affected in CF MDMs, are unknown.

4 ADDITIONAL ELIGIBILITY CRITERIA

Only participants enrolled at Queensland FOR*Ma*T trial sites will be eligible to participate in sub-study B2.2. Furthermore, samples required for sub-study B2.2 are derived from the whole blood sample obtained in sub-study B2.1. Therefore, only participants enrolled in sub-study B2.1 are eligible for sub-study B2.2. In addition, participants eligibility will be based on the following criteria:

4.1 Inclusion Criteria

- Participant is enrolled at the following sites:
    - Queensland Children's Hospital,
    - The Prince Charles Hospital,
    - Princess Alexandra Hospital,
    - Royal Brisbane and Women's Hospital,
    - Gold Coast University Hospital,
    - Greenslopes Private Hospital,
    - Mater Adults Hospital,
    - Sunshine Coast University Hospital.
- Enrolled in sub-study B2.1.
- Aged six years and older.
- Ability to provide a minimum 5ml whole blood sample (this is for both sub-studies B2.1 – B2.3).
- Enrolled in either the observation or intervention programs.
- Participant is enrolled at a FOR*Ma*T Trial site that can comply with sampling requirements
- Informed consent by participant or participants parent/guardian.

4.2 Exclusion Criteria

No additional exclusionary criteria to those outlined in the FOR*Ma*T Master Protocol apply to sub-study B2.2.

5 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study B2.2, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

6 SUB-STUDY SPECIFIC PROCEDURES

Mitochondrial samples are obtained from the 5-10 ml (age dependent) whole blood samples collected in sub-study B2.1. Refer to the above section for a description of the procedures. No additional samples are required. Trial ID number, date and time of collection are to be recorded on the sample tube and sub-study CRF.

Samples will be collected in accordance with Table 24: Sub-study B2.2 Schedule of Assessments. The collection, transport and storage of samples is in accordance with the macrophage and mitochondrial SOP's described in Appendix D.

Observational cohort participants are required to provided samples at either week 52 and 56 or week 58 and 62, not both.

Table 23: Sub-study B2.2 Schedule of Assessments

ASSESSMENT	**STUDY VISITS**					
	Screening Visit (Day 0)	**Week 6**	**Week 12**	**Week 28**	**End of consolidation***	**Final Outcome^**
Observation Cohort Participant Timeframes	-14 days	+14 Days	±14 Days	±14 Days	NA	±14 Days
Intervention Program Participant Timeframes	-14 Days	±3 Days	±3 Days	±5 Days	+5 Days	+14 Days
Review Eligibility for Sub-Study B2.2	✓					
Mitochondrial Sample	✓	✓	✓	✓	✓	✓

*Intervention Program participants only. For Intervention Program participants randomised to Week short intensive, this is collected at Week 52. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 58.
^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.

7 STATISTICAL ANALYSIS

Based on previous experience, the data generated in these experiments are likely to be non-normally distributed (Shapiro-Wilk test), in which case grouped data will be described by group median (25^{th} and 75^{th} percentile). Comparisons between groups will be undertaken using Mann-Whitney U-Test, Kruskal-Wallis test on ranks (non-parametric alternative to one-way analysis of variance), or Friedman test, as appropriate. Regression analyses will be used to control for likely confounder or modifier variables, including age, sex, previous treatment, current CFT corrector/potentiators therapy. The techniques used for regression analyses will depend on the final; sample size. If large enough, parametric methods will be used as the key assumption is that the residuals of the regression are normally distributed, and the Central Limit Theorem tells us that as the sample size increases all distributions of residuals tend towards normality. If the sample size is not sufficient a multivariable model using quantile regression, in which the median difference and 95% CI between groups can be determined.

SUB-STUDY B2.3: GENE EXPRESSION SIGNATURES

Investigator Lead

Associate Professor Lachlan Coin

1 PRIMARY OBJECTIVE

Identify biomarker signatures for MABS clearance at the end of intensive therapy and at the final outcome, distinguishing MABS colonisation from MABS-PD between the observational cohort and intervention program participants.

2 INTRODUCTION

In addition to serology biomarkers, development of gene expression signatures may provide an important adjunct for diagnosing and monitoring MABS infection, disease progression and response to treatment. High quality evidence obtained from the FOR*Ma*T trial will guide decisions for starting treatment and measuring disease severity in patients with MABS-PD, influencing global practice.

Additional Eligibility Criteria

All observation and intervention program participants are eligible to participate in sub-study B2.3 in accordance with the FOR*Ma*T Master Protocol eligibility. In addition, participants are required to meet the following criteria:

2.1 Inclusion Criteria

- Ability to provide a 2.5ml (participants <18 years of age) and 10ml (participants ≥18 years of age) whole blood sample.
- Participant is enrolled at a FOR*Ma*T Trial site that can comply with sampling requirements.
- Enrolled in either the observation or intervention programs.
- Informed consent by participant or participants parent/guardian.

2.2 Exclusion Criteria

No additional exclusion criteria apply.

3 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study B2.3, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

4 SUB-STUDY SPECIFIC PROCEDURES

Whole blood samples of 2.5ml (<18 years of age) and 10ml (≥18 years of age) in PAXgene tubes, optimised for RNA will be collected. Whole blood samples for gene expression signature biomarkers

will be collected in accordance with Table 25, Sub-Study B2.3 Schedule of Assessments. Trial ID number, date and time of collection are to be recorded on the sample tube and sub-study CRF. Blood RNA Tubes are to be transported to the central study laboratory and shipped to Queensland Paediatric Infectious Diseases Laboratory at QCH, for longer term storage at -80°C prior to batch shipping the samples to the Peter Doherty Institute, University of Melbourne. Alternatively, the samples can be sent directly to the Peter Doherty Institute, University of Melbourne, Australia for processing. Collection, storage and transport of all samples will be made in accordance with Appendix D, Gene Expression Signature SOPs.

Observational cohort participants are required to provided samples at either week 52 and 56 or week 58 and 62, not both.

Table 24: Sub-study B2.3 Schedule of Assessments

ASSESSMENT	**STUDY VISITS**		
	Screening Visit (Day 0)	**Week 6**	**Final Outcome^**
Observation Cohort Participant Timeframes	-14 days	+14 Days	±14 Days
Intervention Program Participant Timeframes	-14 Days	±3 Days	+14 Days
Review Eligibility for Sub-Study B2.3	✓		
Gene Expression Signatures	✓	✓	✓

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.

5 STATISTICAL ANALYSIS

5.1 Identifying a biomarker signature for MABS-PD microbiological cure (clearance)

RNAseq will be conducted on bloods taken at baseline and at their final outcome assessment, generating ~30m reads per sample. We will use Salmon (85) to calculate transcript abundance from these data, and Limma-Voom pipeline (86) to identify statistically significant differences in expressed transcripts between acute and convalescent time-points. Using the ssizeRNA package in R we can estimate that this approach provides 99.5% power at a FDR of 5% to identify genes with a 1.4 fold-change between conditions, and 50% power at a fold-change of 1.2. We will use the elastic net (87) to identify a biomarker signature for predicting recovery, as well as the FS-PLS algorithm we have described

previously for identifying minimal biomarker signatures of recovery (88). Identified biomarkers will be measured on the intermediate time-points, using nanoString platform in order to investigate the variability of the biomarker signature over time. The identified biomarkers will be validated using the nanoString platform on blood collected from participants with *M. abscessus* infection recruited in years 3 and 4 of the trial.

5.2 Identifying a biomarker signature of MABS-PD vs simple colonisation:

RNAseq will be performed on blood collected from 100 patients recruited into the observational cohort during the first three years of the trial. Approximately half of these are expected to later develop MABS-PD. As above, we will use Salmon (85) to quantify expression, and Limma-Voom (86) to identify significant differences in expressed transcripts between these two groups, as well as the elastic net (87) and FS-PLS to identify biomarkers of infection vs colonisation. These signatures will be validated using nanoString on a sample of a further 100 samples collected during study years 3 and 4. As above, this approach has a 99.5% power to identify genes with a 1.4 fold-change.

SUB-STUDY B2.4: T-CELL FUNCTION

Investigator Leads

Associate Professor John Miles and Associate Professor David Reid

1- PRIMARY OBJECTIVE

Determine susceptibility to MABS infection in patients with MABS-PD by examining T-cell function in response to infection compared with participants enrolled in the observational cohort.

2 INTRODUCTION

T cells play a critical role in MABS sensing and MABS clearance. It has been hypothesized that CF patients susceptible to MABS infection may have one or more failures in immune control. Using high dimensional immunoprofiling, we recently validated this hypothesis and found CF patients with current or past MABS infection showed significant distortions in T cell subtypes and in T cell function32. Ex vivo differences in Treg percentages and in T cell activation (CD25) and exhaustion (CTLA-4 & PD-1) markers were observed. As were, differences in polyfunctionality of CD4+ T cells (TNF-α, INF-γ & IL-2), revealing 17 new immune biomarkers for MABS susceptibility. Using these new immune biomarkers, a regression model was generated that produced an AUC of 1. Notably, TNF-α secretion appeared to be defective in CD4+ T cells of CF patients susceptible to MABS-PD infection. This is important given TNF-α directly activates macrophages to restrict mycobacterial growth and induces apoptosis of infected macrophages leading to bacterial killing (89). TNF-α is also essential for granuloma formation and disease restriction (90) and mice deficient in TNF-α are highly susceptible to disseminated forms of mycobacteria (91). This published data around immune failures in CF patients susceptible to MABS could point the way to immune modulating therapies to complement antibiotics and possibly predict therapeutic outcome, but this remains speculative.

The FOR*Ma*T trial provides a unique opportunity to examine T cell phenotype in a much larger population of individuals with and without CF than has been previously possible. The projected number of new MABS-PD diagnoses and the participants enrolled in the observational platform will allow a comprehensive study of T cell function and allow the conduct of the critical functional experiments that are required to pave the way for new immunomodulating therapeutics and clinical biomarkers.

3 ADDITIONAL ELIGIBILITY CRITERIA

Only participants enrolled at Queensland FOR*Ma*T trial sites and in sub-study B2.1 and B2.3 will be eligible to participate in sub-study B2.4. This is because the samples required for sub-study B2.4 are derived from the samples obtained in sub-study B2.1 and B2.3. These criteria are reflected below:

3.1 Inclusion Criteria

- Participant is enrolled at the following sites:
    - Queensland Children's Hospital,
    - The Prince Charles Hospital,
    - Princess Alexandra Hospital,
    - Royal Brisbane and Women's Hospital,
    - Gold Coast University Hospital,
    - Greenslopes Private Hospital,
    - Mater Adults Hospital,
    - Sunshine Coast University Hospital.
- Aged six years and older.
- Enrolled in sub-study B2.1 and B2.3.
- Ability to provide a minimum 5ml whole blood sample.
- Enrolled in either the observation or intervention programs.
- Informed consent by participant or participants parent/guardian.

3.2 Exclusion Criteria

No additional exclusionary criteria to those outlined in the FOR*Ma*T Master Protocol apply to sub-study B2.4.

4 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study B2.4, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

5 SUB-STUDY SPECIFIC PROCEDURES

2 x 10^6 peripheral blood mononuclear cells (PBMCs)/ml and 5ml of plasma will be separated from the whole blood sample provided in sub-study B2.1. No additional samples are required. Trial ID number, date and time of collection are to be recorded on the sample tube and sub-study CRF. All samples will be processed at the CCHR, South Brisbane. Samples will be batched and sent to James Cook University as requested.

High-dimensional immunoprofiling will be performed (multiparametric flow cytometry & NanoString analysis) on T cell populations from the same participants examined in sub-study B2.3.We will then design custom flow cytometry panels and custom NanoString panels based on the strongest T cell biomarkers identified by RNAseq (A/Prof Coin). Two or more aliquots of PBMCs will be available from each participant. One aliquot will be used for T cell analysis and one aliquot used for macrophage analysis. Multiparametric flow cytometry will be conducted on major T cell subsets, including central

memory (CM), effector memory (EM) and effector memory RA+ (EMRA). Both CD8+ and CD4+ subsets will be profiled along antigen-specific populations using recombinant purified protein derivative (PPD) as a stimulus. NanoString analysis will be performed based on gene signatures CD8+ and CD4+ subsets as we have previously described (92).

Samples will be collected in accordance with Table 26: Sub-study B2.4 Schedule of Assessments. The collection, transport and storage of samples is in accordance with the T-cell function SOP's described in Appendix D.

Observational cohort participants are required to provided samples at either week 52 and 56 or week 58 and 62, not both.

Table 25: Sub-study B2.4 Schedule of Assessments

ASSESSMENT	STUDY VISITS					
	Screening Visit (Day 0)	**Week 6**	**Week 12**	**Week 28**	**End of consolidation***	**Final Outcome^**
Observation Cohort Participant Timeframes	-14 days	+14 Days	±14 Days	±14 Days	NA	±14 Days
Intervention Program Participant Timeframes	-14 Days	±3 Days	±3 Days	±5 Days	+5 Days	+14 Days
Review Eligibility for Sub-Study B2.4	✓					
T-Cell Sample~	✓	✓	✓	✓	✓	✓

*Intervention Program participants only. For Intervention Program participants randomised to Week short intensive, this is collected at Week 52. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 58.
^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.
~T-cell samples will be obtained from the whole blood sample collected in sub-study B2.1. No additional blood samples are required.

6 STATISTICAL ANALYSIS

Flow cytometry data will be analysed using Cytobank which will include heatmap, SPADE, viSNE, dot and histogram overlays and CITRUS outputs. NanoString data will be analysed using nSolver and Gmine. Gmine analysis will include heatmap, multivariate methods including principal component analysis (PCA), redundancy analysis (RDA) and canonical correspondence analysis (CCA), network analysis, biomarker discovery and multivariable linear regression analysis.

SUB-STUDY B2.5: SEROLOGY FOR MABS

Investigator Leads

Professor Niels Høiby and Dr Tavs Qvist

1 PRIMARY OBJECTIVE

Identify serological markers that predict 1) the natural history of MABS colonisation and 2) progression to MABS-PD as well as 3) treatment failure in different patient subpopulations.

2 INTRODUCTION

Targeted therapeutic approaches to maximise clinical benefit are required in the treatment of MABS-PD. The development of serological assessments to help predict and identify patients who may benefit from early treatment and identify successful responses to treatment is required. In doing so, unnecessary treatment associated toxicity and costs are reduced, improving outcomes for MABS-PD patients.

3 ADDITIONAL ELIGIBILITY CRITERIA

All observational cohort and intervention program participants are eligible to participate in sub-study B2.5 in accordance with the FOR*Ma*T Master Protocol eligibility. In addition, participants are required to meet the following criteria:

3.1 Inclusion Criteria

- Ability to provide a minimum 1ml serum sample.
- Enrolled at a FOR*Ma*T Trial site that can comply with sampling requirements.
- Enrolled in either the observational cohort or intervention program.
- Informed consent by participant or participants parent/guardian.

3.2 Exclusion Criteria

No additional exclusion criteria apply.

4 ADDITIONAL INFORMED CONSENT REQUIREMENTS

Additional blood samples are required as part of the study procedures for sub-study B2.5, therefore additional consent will be obtained in accordance with the FOR*Ma*T Master Protocol.

5 SUB-STUDY SPECIFIC PROCEDURES

1ml of serum is obtained by venepuncture and left to clot at ambient temperature. Centrifuging is carried out to pellet the blood cells and serum is then collected by pipetting to a plastic tube (e.g. NAGLE no. 479-3222 from VWR) with an airtight screw cap. Serum samples will be collected in accordance with Table 27, Sub-Study B2.5 Schedule of Assessments. Trial ID number, date and time of collection are

to be recorded on the sample tube and sub-study CRF. Once the sample is processed the tube containing the sample can be stored on site at -20°C. If -20°C storage is not available onsite samples can be transported to CCHR. All serum samples will be batched and shipped to the Department of Clinical Microbiology, Copenhagen regularly (approximately every three months), although this may be more or less frequent depending on recruitment and site storage ability. Collection, storage and transport of all serum samples will be made in accordance with Appendix D.

Observational cohort participants are required to provided samples at either week 52 and 56 or week 58 and 62, not both.

Table 26: Sub-study B2.5 Schedule of Assessments

ASSESSMENT	STUDY VISITS					
	Screening Visit (Day 0)	**Week 6**	**Week 12**	**Week 28**	**End of consolidation***	**Final Outcome^**
Observation Cohort Participant Timeframes	-14 days	+14 Days	±14 Days	±14 Days	NA	±14 Days
Intervention Program Participant Timeframes	-14 Days	±3 Days	±3 Days	±5 Days	+5 Days	+14 Days
Review Eligibility for Sub-Study B2.5	✓					
Serology	✓	✓	✓	✓	✓	✓

*Intervention Program participants only. For Intervention Program participants randomised to Week short intensive, this is collected at Week 52. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 58.
^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.

6 STATISTICAL ANALYSIS

Analysis will be descriptive and examine changes across both successful and failed treatment courses in the different subgroups and differences between those who develop MABS-PD and those who are colonized. Validation studies will be developed in the future.

Baseline data will be reported as median and interquartile range (IQR) for non-normally distributed continuous variables, and as percentages for categorical variables. Group comparisons will be made using Kruskal-Wallis test on ranks (non-parametric alternative to one-way analysis of variance) and Dunn's multiple comparison test. A p-value of ≤ 0.05 will be considered statistically significant. Longitudinal anti-MABS IgG kinetics during the intervention program will be compared to the

observational cohort and effect of treatment will be assessed. A diagnostic algorithm will be constructed based on the principle of risk stratification. The premise of the algorithm will be the predictive value of one routine serum sample for anti-MABS per patient per year. Multilevel predictive values, likelihood ratios and a receiver operating curve for sensitivity and specificity will be constructed. All measures will be reported with confidence intervals. Participants will be divided into phenotype groups on the basis of NTM culture results and clinical data captured from patient records:

1. Participants with MABS-PD disease who are not receiving treatment (at enrolment in the intervention trial or remaining in the observation cohort without treatment).
2. Those that clear infection with treatment during FOR*Ma*T.
3. Those that do not clear infection with treatment during FOR*Ma*T.
4. Participants with positive MABS cultures but no pulmonary disease in the observation cohort.
5. Control samples will also be available from patients with no known history of NTM infection and current negative NTM culture results. These control samples are available through samples currently being collected as part of other clinical studies: (CF control samples- Early Life Origins of CF lung disease CFFT grant PI Sly 2019-2021) that includes samples from adults and children (estimated 200 blood samples will be available matched with NTM cultures performed and full clinical history). Non- CF control samples will be sourced from a study in adult patients being investigated for respiratory disease by the team led by Professor Peter Wark (site investigator for FOR*Ma*T) at John Hunter Hospital, Newcastle Australia and University of Newcastle. These patients will have available clinical information, chest CT scans, three sputum cultures or BAL that will confirm they are not infected with NTM. An estimated 20-30 samples would be available from this study.

Recognised previous NTM infections will be documented at enrolment in the FOR*Ma*T intervention trial and observation cohort and any new NTM infections detected during the study will also be documented. The presence of past or present infection with another NTM on MABS serology will be examined.

Coefficient of variance will be reported. The study will be reported in accordance with the guidelines for reporting studies on diagnostic accuracy (STARD).

SUMMARY OF SAMPLE COLLECTION

Table 27: Sample requirements for B1 sub-studies

Assessment	Sample and Volume Needed	Study Visit					
		Screening Visit (Day 0)	Week 6	Week 12	Week 28	End of consolidation*	Final Outcome^
Observation Cohort Participant Timeframes	NA	-14 days	+14 Days	±14 Days	±14 Days	NA	±14 Days
Intervention Program Participant Timeframes	NA	-14 Days	±3 Days	±3 Days	±5 Days	+5 Days	+14 Days
Review Eligibility for Sub-Studies	NA	✓					
Macrophage and Mitochondrial Samples	5ml (6-11y) 10ml (12+y)	✓	✓	✓	✓	✓	✓
T-cells	5ml plasma PBMCs are from macro sample	✓	✓	✓	✓	✓	✓
Serology	1ml serum	✓	✓	✓	✓	✓	✓
Gene Expression Signatures	2.5ml whole blood (<18y) 10ml (>18y)	✓	✓	-	-	-	✓

*****Intervention Program participants only. For Intervention Program participants randomised to Week short intensive, this is collected at Week 52. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 58.

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.

APPENDIX B3: IMAGING

Investigator Leads

Professor Harm Tiddens

1 PRIMARY OBJECTIVE:

To examine the changes in structural lung disease as detected on chest CT scan (PRAGMA % disease, bronchiectasis, mucus plugging and trapped air) across the FOR*Ma*T intervention program associated with and without clearance of MABS at 4 and 12 weeks and at final outcome.

2 SECONDARY OBJECTIVES:

1. Compare the progression of structural changes on chest CT scan (PRAGMA % disease, bronchiectasis, mucus plugging and trapped air) in patients in the intervention program and patients in the observational cohort with and without MABS-PD.
2. Compare progression of structural changes on chest CT scan (PRAGMA % disease, bronchiectasis, mucus plugging and trapped air) associated with the development of MABS-PD.
3. Develop a scoring system for chest CT imaging of MABS-PD infection.
4. Identify CT related biomarkers to predict treatment response.

3 INTRODUCTION

CT scans are regarded as important components of establishing the diagnosis of MABS-PD according to the ATS criteria and are recommended in international guidelines as part of the assessments to be undertaken when starting and ending treatment in patients with MABS-PD (1). There is also some evidence that patients who clear MAC infection have improved radiological outcomes compared with those who do not clear infection (93) and that early radiological improvement is a predictor of converting to negative sputum cultures. In this study a relative crude image analysis was used, furthermore image acquisition was not standardized. Hence, it is likely that chest CT outcomes using standardized chest CT protocols and more sensitive image analysis systems such as PRAGMA-CF can improve the predictive power for sputum conversion and radiological resolution of MABS-PD related structural changes. This trial provides an opportunity to include standardized chest CT scan according to ATS criteria for MABS-PD for phenotyping of MABS-PD patients.

Using CT related phenotyping the FOR*Ma*T trial provides an opportunity to document the spectrum and changes in structural lung disease as detected on chest CT scan in patients with MABS who do or do not clear infection as well as progression in those with positive MABS cultures that do and do not develop MABS-PD. From a safety and clinical perspective, MABS infection may be cleared

from the sputum but this response might be dissociated from progression of structural lung disease as it is questionable whether sputum is reflective of consolidated lung areas.

There are no specific scoring systems for MABS-PD and the FOR*Ma*T trial provide a unique opportunity for the development of a radiological scoring system specifically for patients with MABS who do or do not have CF and this will be part of the Discovery studies planned. PRAGMA-CF is currently being automated for monitoring CF lung disease. For FOR*Ma*T all CTs need to be manually annotated. Next these annotated CTs can be used to adjust and train the algorithm future automated image analysis of MABS patients.

4 ADDITIONAL ELIGIBILITY CRITERIA

No additional eligibility criteria to those outlined in the FOR*Ma*T Master Protocol apply to sub-study B3.1.

5 ADDITIONAL INFORMED CONSENT

All CT scans are performed as part of the FOR*Ma*T Master Protocol. Additional consent is required for the chest CT scan at 12 weeks, see Appendix D.

6 CT TRIAL PROCEDURES

An inspiratory and expiratory helical chest CT scan with HRCT reconstruction will be performed for all FOR*Ma*T trial participants in accordance with the core trial procedures, Table 1.

Scans will be performed using optimization of lung volumes to provide standardization longitudinally. Each FOR*Ma*T site will have a CT scanning protocol developed, taking into account site specific scanning requirements optimising the balance between radiation and image quality. Site radiology staff will undergo web-based training and standardization of CT protocols undertaken by the Erasmus team. All images will be de-identified and sent electronically to Erasmus MC for scoring using the PRAGMA scoring system (see imaging SOPs Appendix D).

7 CT SCORING

All scans will be manually scored using the PRAGMA-CF scoring system which has been extensively validated for quantitative analysis of CF lung disease in children and adolescents (94, 95). Compared with other scoring systems, PRAGMA has increased sensitivity for detecting and monitoring changes in mild as well as in advanced disease (including inflammatory changes, such as mucus plugging, “tree-in-bud”, consolidation and bronchiectasis seen in MABS-PD) and has a known expected change with time for CF, which is important for maintaining discriminatory ability and sensitivity to potential change from additional disease (MABS-PD). For example, PRAGMA-

CF has been used as outcome measure in a large Phase III clinical trial of Ataluren in 207 children and adults with CF. Longitudinal analysis showed that %Disease at start of study was 9.8% and that it progressed significantly over the study duration (pers. com Prof Harm Tiddens, paper submitted). No difference was detected between the ataluren and placebo group. This lack of effectiveness was later confirmed in a second Ataluren phase III trial and used in a Phase III clinical trial of Ataluren.

Scoring will be undertaken blinded to treatment allocation and clinical data by the Erasmus MC LungAnalysis team.

Numbers of available scans:

There will be 300 paired scans (N=600) from enrolment to completion of the FOR*Ma*T trial for the initial Appendix A1 intervention trial with 150 pairs from participants with CF and 150 pairs from participants without CF. We estimate from discussions with patients and from other studies that approximately 200 participants will consent to a further scan at 12 weeks. The risk related to the extra radiation of the third CT scan is low and is estimated to be in the order of 3-6 months background radiation (96). New MABS notifications in Queensland Australia now average >100/year (≈1/3rd CF). An audit of 51 adults with CF and MABS infection in Queensland found 42% did not receive treatment and so would potentially enter the observation arm, although reasons for this were variable. The Royal Brompton Hospital London (pers. com. Prof Elborn and Dr. Jones) suggested 4/49 (8%) patients in their cohort culturing MABS had single isolates and therefore would not meet ATS criteria but would be eligible for the observation arm. For the observational cohort we estimate that 1/3 of subjects will not meet ATS criteria (estimate 50 paired scans) and 1/3 would start in the observation arm develop MABS-PD and move into the trial subsequently (estimate 50 paired scans) and potentially 1/3 have MABS-PD and not receive treatment through choice (estimate 50 paired scans). In an observation study with 180 subjects 82% of subjects had sputum conversion of MAC on treatment and this was associated with a significant improvement ($P<0.0001$) in structural lung disease (93). This suggests that the numbers of scans available in this trial will support the planned analyses.

SUB-STUDY B4: VESTIBULAR

Investigator Lead

Gretta Palmer

1 PRIMARY OBJECTIVE

Utility of vestibular screening tests in the detection of vestibulotoxicity related to prolonged administration of aminoglycosides for individuals with *Mycobacterium abscessus* pulmonary disease (MABS-PD).

2 SECONDARY OBJECTIVES

1. To determine the sensitivity and specificity of non-invasive vestibular screening tests to detect vestibulotoxicity in patients with *Mycobacterium abscessus* pulmonary disease receiving prolonged aminoglycosides.
2. To determine the i) proportion; and ii) time to onset of vestibulotoxicity in patients with *Mycobacterium abscessus* pulmonary disease receiving prolonged aminoglycosides from Day 1 to one-month post treatment (either week 56 or 62, depending on how the participant is randomised throughout the trial). weeks.
3. To explore the association between the presence of symptoms and the time to onset of vestibulotoxicity in patients with non-tuberculosis mycobacteria receiving prolonged aminoglycosides.
4. To explore the association between clinical factors (sociodemographic, treatment-related) and time to onset of vestibulotoxicity in patients with non-tuberculosis mycobacteria receiving prolonged aminoglycosides.

3 INTRODUCTION

It has been well documented that aminoglycoside antibiotics can have a toxic effect to the inner ear hair cells of both the cochlear and vestibular systems and hence they can cause disorders of hearing and balance respectively. Vestibulotoxicity can exist independently from cochlear toxicity. Vestibulotoxicity is the term used to describe toxicity caused by preferential impairment of all vestibular end organs of the inner ear, namely the semicircular canals and otolith organs.

Symptoms and signs of bilateral vestibulopathy occur due to the bilaterally reduced or absent vestibulo-ocular reflex (VOR). Therefore, vestibulotoxicity typically manifests with significant postural instability and unsteadiness of gait and motion induced oscillopsia or visual blurring with head movement such as walking. Frequently permanent, vestibulotoxicity can result in a severe, bilateral vestibular loss causing debilitating imbalance resulting in significant falls risk, failure to return to work and reduced quality of life.

Unfortunately, vestibulotoxicity cannot typically be remedied easily or fully by therapeutic intervention or rehabilitated with Vestibular Rehabilitation and Balance Therapy (VRBT), which is why a discussion of vestibulotoxicity and tests to screen for the early detection of it is of great importance.

There are few studies investigating the methods to screen patients for vestibulotoxicity who have been exposed to prolonged aminoglycosides. Moreover, the current gold standard tests used to diagnose vestibulotoxicity are only available in specialist departments. These tests often require expensive equipment, extensive staff training and clinical expertise to interpret the results. In addition, these tests can be somewhat invasive and may temporarily cause or exacerbate pre-existing dizziness symptoms further adding to the patient's treatment burden.

The findings from this sub-study will inform the development of best practice guidelines for the vestibular monitoring of patients with non-tuberculosis mycobacteria receiving prolonged aminoglycosides.

4 ADDITIONAL ELIGIBILITY CRITERIA

In addition to meeting the eligibility criteria described in the Master Protocol, participants are required to meet the following eligibility criteria:

4.1 Additional Inclusion Criteria

- Enrolled in the intervention cohort at one of the following sites:
    - Queensland Children's Hospital,
    - The Prince Charles Hospital,
    - Princess Alexandra Hospital,
    - Royal Brisbane and Women's Hospital,
    - Gold Coast University Hospital,
    - Sunshine Coast University Hospital,
    - Mater Adult's Hospital,
    - Sunshine Coast University Hospital,
    - Cairns Base Hospital,
    - Townsville Base Hospital,
    - Logan Hospital,
    - Mackay Base Hospital.
- Participant is aged 6 years and older.
- Participant is enrolled at a FOR*Ma*T trial site that has a Vestibular Physiotherapist who can comply with the vestibular monitoring schedule.
- Informed consent by participant or participants parent/guardian.

4.2 Additional Exclusion Criteria

- Patients who have contraindications to or are unable to undergo bedside testing and formal audio-vestibular testing such as with caloric testing (e.g. prior tympanic membrane rupture) or to the head impulse test (e.g. significant neck immobility or pain).
- Participants enrolled in the observation program.

5 ADDITIONAL INFORMED CONSENT

Vestibular testing in addition to that in the main study is required as part of the sub-study procedures for sub-study B4, with caloric testing via Audiology and video Head Impulse Testing (vHIT) primarily via Physiotherapy (but may be done via Audiology as some sites); therefore additional consent for this sub-study will be obtained in accordance with the FOR*Ma*T Master Protocol.

6 SUB-STUDY SPECIFIC PROCEDURES

Participants will be assessed by a Vestibular Physiotherapist and vestibular testing completed in accordance with the schedule of assessments in Table 28. At each assessment, a Vestibular Physiotherapist will administer the Vestibulotoxicity Screening Questionnaire (Table 29) and a series of bedside vestibular examination tests. A verbal explanation of each clinical test will be provided to participants throughout the physical assessment. Caloric testing (the gold standard for assessing vestibulotoxicity) with an audiologist will be measured at screening and at the final study visit, either week 56 or 62 depending on the participants randomisation during the trial.

Table 28: Sub-study B2.5 Schedule of Assessments

Assessment	Study Visit									
	Screening Visit (Day 0)	**Wk 2**	**Wk 4**	**Wk 6**	**Wk 8**	**Wk 10**	**Wk 12**	**Wk 28**	**End of consolidation***	**Final Outcome^**
Intervention Program Participant Timeframes	-14 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±3 Days	±5 Days	+5 Days	+14 Days
Review Eligibility for Sub-Studies	✓									
Vestibular testing; HIT +/- Vhit, DVA and Romberg foam	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Audiogram	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Calorics	✓								✓	✓

*For Intervention Program participants randomised to Week short intensive, this is collected at Week 52. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 58.

^For Observation Cohort participants and Intervention Program participants randomised to short intensive, this is collected at Week 56. For Intervention Program participants randomised to prolonged intensive, this is collected at Week 62.

Table 29: Vestibulotoxicity Screening Questionnaire

These questions are about symptoms that you, or your child may be experiencing.

		Yes	No
Q1.	In the last 7 days, have you had a feeling that your vision is not stable and steady (bobbing/ bouncing/ slipping/ blurring) **when you are moving your head**?		
Q2.	In the last 7 days, have you had a feeling that your vision is not stable and steady (bobbing/ bouncing/ slipping/ blurring) **when you are walking around**?		
Q3.	In the last 7 days, have you experienced a recent onset of **imbalance, falls or near misses**?		

If you answered yes to any of the above symptoms:

		Yes	No
Q4.	Are your symptoms **present when standing and walking** but **absent when sitting and lying down**?		
Q5.	Do your symptoms increase when **walking in the dark or on uneven ground**?		

Regarding your hearing:

		Yes	No
Q6.	In the last 7 days, have you noticed any onset or significant worsening of **hearing loss** in one or both ears?		
Q7.	In the last 7 days, have you noticed any onset or significant worsening of **tinnitus (ringing in the ears)** in one or both ears?		

7 VESTIBULAR TESTING

The clinical diagnosis of bilateral vestibular loss will be based on the results of these three simple non-invasive bedside tests:

1) the Romberg on Foam test;

2) the Head Impulse Test (HIT) and;

3) the Dynamic Visual Acuity (DVA) test.

If available at the specific site, the Vestibular Physiotherapist will directly measure and quantify the vestibulo-ocular reflex (VOR) deficit using a portable lightweight video Head Impulse Test (vHIT) device, which has been shown to be more sensitive and specific than the bedside HIT.

7.1 Romberg on Foam

The participant will be asked to stand on a mat of rubber foam (standardised structural PVC foam 6cm thick) (e.g. HART Sport Foam Balance Pad 38 cm x 48cm x 6cm) with feet together, first with eyes open and then closed. During the Romberg on foam test of balance, the Vestibular Physiotherapist will stand within close proximity to the participant to minimise the risk of falling. The participants will be in control of their own active movement i.e. no external force will be applied.

The Vestibular Physiotherapist will record the total time (manually by stopwatch in seconds) the participant can maintain the feet together/eyes open standing on foam position, up to a maximum of 30 seconds. There will be three trials conducted. The participant will then be asked to stand with their eyes closed and the test will be repeated. This will also be repeated three times.

7.2 Head Impulse Test (HIT)

The manual HIT will be performed with the participant in a seated position. The Vestibular Physiotherapist will ask the participant to focus on a target (e.g. the tip of Vestibular Physiotherapist's nose). The Vestibular Physiotherapist will then manually deliver a brisk, passive horizontal head rotation of approximately 10 – 20 degrees amplitude, whilst observing whether or not the eyes of the participant are able to maintain fixation on the target. The HIT will be applied with a maximum of 5 head thrusts to each side (left, right). The Vestibular Physiotherapist will record a positive or negative result (see Section 8.3).

7.3 Dynamic Visual Acuity (DVA)

To conduct the DVA test, the participant will be seated in a well-lit room, 4 feet (1.2m) opposite a wall, on which hangs a copy of the ETDRS A4 printable visual acuity chart (or appropriate distance away using the Snellen chart). Participants may wear glasses if they require them, and the presence of glasses will be noted. Participants will be asked to read the visual acuity chart with their head stationary. The Vestibular Physiotherapist will record the line in which the participant first makes an error (says 3 or

more incorrect letters in a line), and the test is ended. The Vestibular Physiotherapist will then manually oscillate the participant's head horizontally and passively at 2Hz (2 full cycles side to side) and ask the participant to read the chart again. The Vestibular Physiotherapist will record the line in which the participant first makes an error (says 3 or more incorrect letters in a line) or resists the passive neck movement and the test is ended.

7.4 Video Head Impulse Test (vHIT)

Some sites at which this sub-study is being conducted will have access to vHIT equipment. If present, this test will be conducted with participants using the same system of vHIT equipment (either Otometrics or Interacoustics) at each assessment point. Participants will don the vHIT lightweight goggles and the Vestibular Physiotherapist will calibrate the equipment. The goggles record eye movements, and the data is captured by the associated computer software program connected to the goggles via cable. The participant will be asked to focus on a fixation dot on the wall at least 1m in front of them. The Vestibular Physiotherapist will apply a manual head rotation (head impulse) similar to the manual HIT test, to each side, until a minimum of ten traces have been accepted for both the left and right horizontal and vertical semicircular canals, with a spread of head velocities across the range from 150 to 300deg/s.

7.5 Caloric testing

To confirm the diagnosis and quantify the vestibular loss, patients will have caloric testing completed at baseline and post treatment. The caloric test will be ordinarily performed with the subject reclining, head inclined 30 deg up from horizontal, so as to make the lateral canal vertical. A warm or cold stimulus is introduced into the ear canal on one side (most commonly air, but water calorics may be used in some clinics). The air is stopped after 60 seconds (water after 30 sec), and any nystagmus elicited is recorded using video-nystagmography (VNG) goggles, with the subject's vision denied and while they perform a mental task (such as naming of animals, counting backwards, etc).

Nystagmus (involuntary eye movement), commonly builds to a peak point after ~ 90 seconds, at which point a fixation target (eg. light) is used to assess fixation suppression. After a rest of at least 3 - 5 minutes, the procedure is repeated on the opposite ear, or with the opposite temperature water.

If little or no nystagmus is elicited using standard air (or water) calorics (a.SPV < 6°/s per irrigation and/or a.SPV < 12°/s total for each ear), ice water (~ 0°/s) must be used for additional irrigations in both ears to determine if a stronger response is able to be elicited or confirm a bilateral weakness.

8 OUTCOME MEASURES

8.1 Romberg on Foam

The Romberg on Foam test will be recorded by measuring the time to failure manually by stopwatch in seconds. Test failure will be defined as:

1) a participant needing to open their eyes;

2) a participant moving their arms or feet in order to achieve stability, or;

3) a participant beginning to fall or requiring hands on assistance to maintain balance and safety.

Participants with bilateral vestibular loss (vestibulotoxicity) have impaired postural control when visual input is blocked and proprioceptive input is disrupted because of reduced vestibulo-spinal reflexes.

The Romberg on foam test will be scored on a Positive (fail) or Negative (pass) basis. Participants will have a positive test for the Romberg on Foam if their average time to failure with eyes closed is significantly less (more than 50%) than their time to failure with eyes open. Participants will have a positive test for the Romberg on Foam if they are deemed to have failed the test (score = 1, 2 or 3) when standing with eyes closed, after having successfully completed the test with eyes open (successfully completed = reached 30s in one of three trials).

8.2 DVA

A positive test result for the DVA will be defined as a difference of more than 2 lines of text between reading with a static head posture and dynamic head movement. In patients with bilateral vestibular loss (vestibulotoxicity), the VOR is no longer able to stabilise gaze when the head is moving fast and as a consequence, visual acuity decreases, and participants will make errors sooner in the text on the chart than when the head is stationary.

8.3 HIT

A positive test result for the HIT will be defined as the presence of an overt corrective catch up saccade (either unilaterally or bilaterally). In a participant with bilateral vestibular loss (vestibulotoxicity), the eyes will move with the head during head impulses to both sides, so that the participant will have to make catch-up saccades after the head movements to bring the eyes back on target.

8.4 vHIT

A positive test result for vHIT will be defined as the presence of catch up saccades and significantly reduced VOR gains (either unilaterally or bilaterally). The diagnosis of bilateral vestibulopathy requires bilaterally significantly impaired or absent function of the VOR. For the diagnosis of bilateral vestibular loss, the horizontal angular VOR gain on both sides should be < 0.6. (5).

8.5 Caloric testing

A presumptive diagnosis of vestibulotoxicity post-treatment will be determined by absent or reduced caloric response compared with baseline. We will classify diminished caloric responses using the generally accepted criteria of (5) an average slow phase velocity (aSPV) of <6deg/sec for all 4 bithermic irrigations and <12deg/sec total aSPV for each ear as measured using videonystagmography (VNG) and using ice calorics (0 deg C) for the cold irrigation. This measure will be used as the gold standard.

9 STATISTICAL ANALYSIS

9.1 Data Management

Management of data within this sub-study will be consistent with the plans for data management in the FOR*Ma*T Master Protocol. All data from the physical assessments will be recorded on a Data Collection Sheet. The questionnaire will be completed in hard copy. These hard copy materials will be stored in a secure locked cabinet within physiotherapy staff offices at each site. Electronic data will be accessible only to the collaborators recorded on the project record and will be only accessible via their institutional usernames and passwords.

9.2 Data Analysis

Analyses for this sub-study will be conducted in SPSS (SPSS Inc., Illinois, USA) or STATA (StataCorp, Texas, USA). Sociodemographic, medical, and other relevant information about participants in the vestibular sub-study will be securely made available to the vestibular sub-study investigators by the FOR*Ma*T trial investigators. Summary statistics (mean, standard deviation, frequency, percentage) will be calculated for the baseline characteristics and outcomes collected in this sub-study.

To asses secondary objective 1, participants that demonstrated absent or reduced caloric response on bithermic caloric testing at the final outcome study visit will be considered as having a diagnosis of vestibulotoxicity (the ‘true condition’). The occurrence of a positive result in each vestibular test (HIT, DVA, Romberg on Foam, +/- vHIT if available) at the final outcome, referred to as the ‘predicted condition’, will be compared with a positive result from caloric testing at the final outcome to identify specificity and sensitivity.

		True condition	
		Condition positive	Condition negative
Predicted condition	Predicted condition positive	True positive	False positive
	Predicted condition negative	False negative	True negative

Sensitivity = number of true positives / (number of true positives + number of false negatives)

Specificity = number of true negatives / (number of true negatives + number of false positives)

The sensitivity and specificity of each of the predictive tests will be presented along with their 95% confidence intervals (CI). Of note, the results for the vHIT will be presented for the subset of participants at the site where the equipment for this test is available.

To assess secondary objective 2, the proportion of participants with vestibulotoxicity will be calculated for each of the follow up assessment time points for each of the vestibular screening tests. A Kaplan Meier curve will be used to describe the time to onset of vestibulotoxicity and determine the median time to onset of vestibulotoxicity and the rate of development of vestibulotoxicity, censoring participants with no vestibulotoxicity at 62 weeks.

To assess secondary objective 3, univariate survival analyses (proportional hazards regression) will be used to explore the association between the presence of symptoms, as identified on the Vestibulotoxicity Screening Questionnaire, and the time to onset of vestibulotoxicity (dependent variable). Results will be presented as hazard ratios (HR) and their 95% CIs.

To assess secondary objective 4, univariate survival analyses (proportional hazards regression) will be used to explore the association between sociodemographic (age, gender) and treatment-related (type of medication) factors, and the time to onset of vestibulotoxicity (dependent variable). These independent variables will be drawn from the baseline assessment. Results will be presented as HRs and their 95 CIs.

APPENDIX C

REGISTRY LINKAGE

Registries that can track important health outcomes longitudinally provide an opportunity to examine the trajectories of patients prior to entering a trial and also following completion of a trial. Many registries are now being used for tracking specific diseases including cystic fibrosis and non-CF bronchiectasis.

Where a FOR*Ma*T participant is already contributing to a disease registry aligned with the FOR*Ma*T trial, consent would be obtained for data from the registry to be shared with FOR*Ma*T. These data would include demographics and clinical information such as nutritional measures, spirometry results and respiratory microbiology results from the previous 5 years as well as up to 5 years post trial participation.

APPENDIX C1: AUSTRALIAN CYSTIC FIBROSIS DATA REGISTRY

Investigator Lead

Dr Susannah Ahern

1 PRIMARY OBJECTIVE

Facilitate linkage between the Australian Cystic Fibrosis Data Registry (ACFDR) and the FOR*Ma*T trial database for data collection and to facilitate examination of longitudinal outcomes before the onset of MABS-PD as well as longitudinal outcomes following completion of trial participation.

2 ADDITIONAL ELIGIBILITY CRITERIA

Additional consent will be sought from FOR*Ma*T trial participants enrolled in the ACFDR to access longitudinal data for five years prior to starting the FOR*Ma*T trial and five years after completing the FOR*Ma*T trial. All observation and intervention program participants are eligible to participate in Appendix C1 if the following criteria are met:

2.1 Inclusion Criteria

- Participant in the FOR*Ma*T Master Protocol.
- Enrolled at an Australian FOR*Ma*T trial site.
- Cystic Fibrosis diagnosis.
- Enrolled in the Australian Cystic Fibrosis Data Registry.
- Informed consent by participant or participants parent/guardian.

2.2 Exclusion Criteria

- Enrolled in a FOR*Ma*T trial site outside of Australia.

3 APPENDIX SPECIFIC PROCEDURES

Data obtained in ACFDR records five years prior to enrolling in the FOR*Ma*T Master Protocol and five years after participating in the FOR*Ma*T trial will be accessed to examine longitudinal data in trial participants. Conversely, data obtained in the FOR*Ma*T trial in consenting participants can be provided to ACFDR to populate the database.

APPENDIX D

STANDARD OPERATING PROCEDURES

Please note: Standard operating procedures have not been included.

REFERENCES

1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, *et al*. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175(4):367-416.
2. Lee MR, Sheng WH, Hung CC, Yu CJ, Lee LN, Hsueh PR. *Mycobacterium abscessus* Complex Infections in Humans. Emerg Infect Dis. 2015;21(9):1638-46.
3. Smibert O, Snell GI, Bills H, Westall GP, Morrissey CO. *Mycobacterium abscessus* Complex - a Particular Challenge in the Setting of Lung Transplantation. Expert Rev Anti Infect Ther. 2016;14(3):325-33.
4. Floto RA, Olivier KN, Saiman L, Daley CL, Herrmann JL, Nick JA, *et al*. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis: executive summary. Thorax. 2016;71(1):88-90.
5. Park IK, Olivier KN. Nontuberculous mycobacteria in cystic fibrosis and non-cystic fibrosis bronchiectasis. Semin Respir Crit Care Med. 2015;36(2):217-24.
6. Olivier KN, Weber DJ, Wallace RJ, Jr., Faiz AR, Lee JH, Zhang Y, *et al.* Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. Am J Respir Crit Care Med. 2003;167(6):828-34.
7. Catherinot E, Roux AL, Vibet MA, Bellis G, Lemonnier L, Le Roux E, *et al*. Inhaled therapies, azithromycin and *Mycobacterium abscessus* in cystic fibrosis patients. Eur Respir J. 2013;41(5):1101-6.
8. Bange, Böttger. Improved Decontamination Method for Recovering Mycobacteria from Patients with Cystic Fibrosis. European Journal of Clinical Microbiology and Infectious Diseases. 2002;21(7):546-8.
9. Ferroni A, Sermet-Gaudelus I, Bourgeois ML, Pierre-Audigier C, Offredo C, Rottman M, *et al*. Measurement of Immunoglobulin G against Mycobacterial Antigen A60 in Patients with Cystic Fibrosis and Lung Infection Due to *Mycobacterium abscessus*. Clin Infect Dis. 2005;40(1):58-66.
10. Stout JE, Floto RA. Treatment of *Mycobacterium abscessus*: all macrolides are equal, but perhaps some are more equal than others. Am J Respir Crit Care Med. 2012;186(9):822-3.
11. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med. 2015;36(1):13-34.
12. Esther CR, Jr., Esserman DA, Gilligan P, Kerr A, Noone PG. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. J Cyst Fibros. 2010;9(2):117-23.
13. Park HY, Jeong BH, Chon HR, Jeon K, Daley CL, Koh WJ. Lung Function Decline According to Clinical Course in Nontuberculous Mycobacterial Lung Disease. Chest. 2016;150(6):1222-32.

14. Diel R, Ringshausen F, Richter E, Welker L, Schmitz J, Nienhaus A. Microbiological and Clinical Outcomes of Treating Non-Mycobacterium Avium Complex Nontuberculous Mycobacterial Pulmonary Disease: A Systematic Review and Meta-Analysis. Chest. 2017;152(1):120-42.
15. Thomson R, Donnan E, Unwin S. Nontuberculous Mycobacterial Lung Disease. Time to Get a Grip! Ann Am Thorac Society. 2015;12(10):1425-7.
16. Qvist T, Taylor-Robinson D, Waldmann E, Olesen HV, Hansen CR, Mathiesen IH, *et al*. Comparing the harmful effects of nontuberculous mycobacteria and Gram negative bacteria on lung function in patients with cystic fibrosis. J Cyst Fibrosis. 2016;15(3):380-5.
17. Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, *et al*. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. Science. 2016;354(6313):751-7.
18. Koh WJ, Jeong BH, Kim SY, Jeon K, Park KU, Jhun BW, *et al*. Mycobacterial Characteristics and Treatment Outcomes in Mycobacterium abscessus Lung Disease. Clin Infect Dis. 2017;64(3):309-16.
19. Rubio M, March F, Garrigo M, Moreno C, Espanol M, Coll P. Inducible and Acquired Clarithromycin Resistance in the *Mycobacterium abscessus* Complex. PloS one. 2015;10(10):e0140166.
20. Park J, Cho J, Lee CH, Han SK, Yim JJ. Progression and Treatment Outcomes of Lung Disease Caused by *Mycobacterium abscessus* and *Mycobacterium massiliense*. Clin Infect Dis. 2017;64(3):301-8.
21. Lyu J, Jang HJ, Song JW, Choi CM, Oh YM, Lee SD, *et al*. Outcomes in patients with *Mycobacterium abscessus* pulmonary disease treated with long-term injectable drugs. Respir Med. 2011;105(5):781-7.
22. Jarand J, Levin A, Zhang L, Huitt G, Mitchell JD, Daley CL. Clinical and microbiologic outcomes in patients receiving treatment for *Mycobacterium abscessus* pulmonary disease. Clin Infect Dis. 2011;52(5):565-71.
23. Aksamit TR, Philley JV, Griffith DE. Nontuberculous mycobacterial (NTM) lung disease: the top ten essentials. Respir Med. 2014;108(3):417-25.
24. Sherrard LJ, Tay GT, Butler CA, Wood ME, Yerkovich S, Ramsay KA, *et al*. Tropical Australia is a potential reservoir of non-tuberculous mycobacteria in cystic fibrosis. Eur Respir J. 2017;49(5).
25. Thomson R, Tolson C, Carter R, Coulter C, Huygens F, Hargreaves M. Isolation of nontuberculous mycobacteria (NTM) from household water and shower aerosols in patients with pulmonary disease caused by NTM. J Clin Microbiol. 2013;51(9):3006-11.
26. Doring G, Flume P, Heijerman H, Elborn JS, Consensus Study G. Treatment of lung infection in patients with cystic fibrosis: current and future strategies. J Cyst Fibros. 2012;11(6):461-79.

27. Lyu J, Kim B-J, Kim B-J, Song JW, Choi C-M, Oh Y-M, *et al*. A shorter treatment duration may be sufficient for patients with *Mycobacterium massiliense* lung disease than with *Mycobacterium abscessus* lung disease. Respir Med. 2014;108(11):1706-12.
28. Waters V, Ratjen F. Antibiotic treatment for nontuberculous mycobacteria lung infection in people with cystic fibrosis. Cochrane Database Syst Rev. 2016;12:CD010004.
29. Novosad SA, Beekmann SE, Polgreen PM, Mackey K, Winthrop KL, Team MaS. Treatment of *Mycobacterium abscessus* Infection. Emerg Infect Dis. 2016;22(3):511-4.
30. Olivier KN, Griffith DE, Eagle G, McGinnis Ii JP, Micioni L, Liu K, *et al*. Randomized Trial of Liposomal Amikacin for Inhalation in Nontuberculous Mycobacterial Lung Disease. Am J Respir Crit Care Med. 2016.
31. Centre for Drug Evaluation and Research US Food and Drug Administration. Non-tuberculous mycobacterial lung infection. Food and Drug Administration (FDA), 2016 April 2016. Report No.
32. Henkle E, Aksamit T, Barker A, Daley CL, Griffith D, Leitman P, *et al*. Patient-Centered Research Priorities for Pulmonary Nontuberculous Mycobacteria (NTM) Infection. An NTM Research Consortium Workshop Report. Ann Am Thorac Society. 2016;13(9):S379-84.
33. Rowbotham NJ, Smith S, Leighton PA, Rayner OC, Gathercole K, Elliott ZC, *et al*. The top 10 research priorities in cystic fibrosis developed by a partnership between people with CF and healthcare providers. Thorax. 2017.
34. Cellamare M, Milstein M, Ventz S, Baudin E, Trippa L, Mitnick CD. Bayesian adaptive randomization in a clinical trial to identify new regimens for MDR-TB: the endTB trial. Int J Tuberc Lung Dis. 2016;20(12):8-12.
35. Berry SM, Connor JT, Lewis RJ. The platform trial: an efficient strategy for evaluating multiple treatments. JAMA. 2015;313(16):1619-20.
36. Bhatt DL, Mehta C. Adaptive Designs for Clinical Trials. NEJM. 2016;375(1):65-74.
37. Davies GR, Phillips PP, Jaki T. Adaptive clinical trials in tuberculosis: applications, challenges and solutions. Int J Tuberc Lung Dis. 2015;19(6):626-34.
38. Fountzilas E, Tsimberidou AM. Overview of precision oncology trials: challenges and opportunities. Expert Rev Clin Pharmacol. 2018;11(8):797-804.
39. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al*. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
40. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, *et al*. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.
41. Smith CL, Jin Y, Raddad E, McNearney TA, Ni X, Monteith D, *et al*. Applications of Bayesian statistical methodology to clinical trial design: A case study of a phase 2 trial with an interim futility assessment in patients with knee osteoarthritis. (Case study). Pharmaceutical Statistics. 2019;18(1):39.

42. Park JJ, Thorlund K, Mills EJ. Critical concepts in adaptive clinical trials. Clin Epidemiol. 2018;10:343-51.
43. Taves DR. Minimization: A new method of assigning patients to treatment and control groups. Clinl Pharmacol Therap. 1974;15(5):443-53.
44. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics. 1975;31(1):103-15.
45. Han B, Enas NH, McEntegart D. Randomization by minimization for unbalanced treatment allocation. Stat Med. 2009;28(27):3329-46.
46. Cuffe RL, Lawrence D, Stone A, Vandemeulebroecke M. When is a seamless study desirable? Case studies from different pharmaceutical sponsors. Pharmaceutical Statistics. 2014;13(4):229-37.
47. Villar SS, Bowden J, Wason J. Response-adaptive designs for binary responses: How to offer patient benefit while being robust to time trends? Pharmaceutical Statistics. 2018;17(2):182-97.
48. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF, *et al*. British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax. 2017;72(Suppl 2).
49. Morisky DE, Ang A, Krousel-Wood M, Ward HJ. Predictive Validity of a Medication Adherence Measure in an Outpatient Setting. J Clin Hypertension. 2008;10(5):348-54.
50. Pearce CJ, Fleming L. Adherence to medication in children and adolescents with asthma: methods for monitoring and intervention. Expert Review Clin Immunol. 2018;14(12):1055-63.
51. Lam WY, Fresco P. Medication adherence measures: an overview. (Report). 2015;2015.
52. Vrijens.B, Urquhart.J. Methods for Measuring, Enhancing, and Accounting for Medication Adherence in Clinical Trials. Clin Pharmacol Therap. 2014;95(6):617.
53. Farmer KC. Methods for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. Clin Therap. 1999;21(6):1074-90.
54. Al-Hassany L, Kloosterboer SM, Dierckx B, Koch B. Assessing methods of measuring medication adherence in chronically ill children-a narrative review. Patient Prefer Adherence. 2019;13:1175-89.
55. Zellner A. An Efficient Method of Estimating Seemingly Unrelated Regressions and Tests for Aggregation Bias. Journal of the American Statistical Association. 1962;57(298):348-68.
56. Glick HA, Doshi JA, Sonnad SS, Polsky D. Economic Evaluation in Clinical Trials: Oxford University Press Catalogue; 2007.
57. Hoch JS, Briggs AH, Willan AR. Something old, something new, something borrowed, something blue: a framework for the marriage of health econometrics and cost-effectiveness analysis. Health Economics. 2002;11(5):415-30.
58. Fenwick E, Byford S. A guide to cost-effectiveness acceptability curves. Br J Psychiatry. 2005;187:106-8.

59. Little RJ, Rubin DB. Statistical Analysis with Missing Data. 2nd edition ed: Wily-Interscience; 2002.
60. Wason JM, Trippa L. A comparison of Bayesian adaptive randomization and multi-stage designs for multi-arm clinical trials. Stat Med. 2014;33(13):2206-21.
61. van Ingen J, Totten SE, Helstrom NK, Heifets LB, Boeree MJ, Daley CL. *In Vitro* Synergy between Clofazimine and Amikacin in Treatment of Nontuberculous Mycobacterial Disease. Antimicrob Agents Chemother. 2012;56(12):6324-7.
62. Shen G-H, Wu B-D, Hu S-T, Lin C-F, Wu K-M, Chen J-H. High efficacy of clofazimine and its synergistic effect with amikacin against rapidly growing mycobacteria. Int J Antimicrob Agents. 2010;35(4):400-4.
63. Harrison MJ, McCarthy M, Fleming C, Hickey C, Shortt C, Eustace JA, *et al*. Inhaled versus nebulised tobramycin: A real world comparison in adult cystic fibrosis (CF). J Cyst Fibros. 2014;13(6):692-8.
64. Nasr SZ, Chou W, Villa KF, Chang E, Broder MS. Adherence to dornase alfa treatment among commercially insured patients with cystic fibrosis. J Med Econom. 2013;16(6):801-8.
65. Bakker EM, Volpi S, Salonini E, van Der Wiel-Kooij EC, Sintnicolaas CJJCM, Hop WCJ, *et al*. Improved treatment response to dornase alfa in cystic fibrosis patients using controlled inhalation. Eur Respir J. 2011;38(6):1328-35.
66. Sturkenboom MGG, Bolhuis MS, Simbar N, Ghimire S, Alffenaar J-WC, Akkerman OW. Amikacin Dosing for MDR Tuberculosis: A Systematic Review to Establish or Revise the Current Recommended Dose for Tuberculosis Treatment. Clin Infect Dis. 2018;67(suppl_3):S303-S7.
67. Whang J, Back YW, Lee KI, Fujiwara N, Paik S, Choi CH, *et al*. *Mycobacterium abscessus* glycopeptidolipids inhibit macrophage apoptosis and bacterial spreading by targeting mitochondrial cyclophilin D. Cell Death Dis. 2017;8(8):e3012.
68. Byrd TF, Lyons CR. Preliminary characterization of a *Mycobacterium abscessus* mutant in human and murine models of infection. Infect Immunity. 1999;67(9):4700-7.
69. Shin DM, Yang CS, Yuk JM, Lee JY, Kim KH, Shin SJ, *et al*. *Mycobacterium abscessus* activates the macrophage innate immune response via a physical and functional interaction between TLR2 and dectin-1. Cell Microbiol. 2008;10(8):1608-21.
70. Lee HM, Yuk JM, Kim KH, Jang J, Kang G, Park JB, *et al*. *Mycobacterium abscessus* activates the NLRP3 inflammasome via Dectin-1-Syk and p62/SQSTM1. Immunol Cell Biol. 2012;90(6):601-10.
71. Wang W, Li T, Wang X, Yuan W, Cheng Y, Zhang H, *et al*. FAM19A4 is a novel cytokine ligand of formyl peptide receptor 1 (FPR1) and is able to promote the migration and phagocytosis of macrophages. Cell Mol Immunol. 2015;12(5):615-24.

72. Di A, Brown ME, Deriy LV, Li C, Szeto FL, Chen Y, *et al*. CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. Nature Cell Biology. 2006;8(9):933-44.
73. Tarique AA, Sly PD, Holt PG, Bosco A, Ware RS, Logan J, *et al*. CFTR-dependent defect in alternatively-activated macrophages in cystic fibrosis. J Cyst Fibros. 2017.
74. Riquelme SA, Hopkins BD, Wolfe AL, DiMango E, Kitur K, Parsons R, *et al*. Cystic Fibrosis Transmembrane Conductance Regulator Attaches Tumor Suppressor PTEN to the Membrane and Promotes Anti *Pseudomonas aeruginosa* Immunity. Immunity. 2017;47(6):1169-81 e7.
75. Bruscia EM, Zhang PX, Ferreira E, Caputo C, Emerson JW, Tuck D, *et al*. Macrophages directly contribute to the exaggerated inflammatory response in cystic fibrosis transmembrane conductance regulator-/- mice. Am J Respir Cell Mol Biol. 2009;40(3):295-304.
76. Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J. Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. Nature. 2002;418(6894):200-3.
77. Tsai RK, Discher DE. Inhibition of "self" engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. J Cell Biol. 2008;180(5):989-1003.
78. Bernut A, Dupont C, Ogryzko NV, Neyret A, Herrmann J-L, Floto RA, *et al*. CFTR Protects against *Mycobacterium abscessus* Infection by Fine-Tuning Host Oxidative Defenses. Cell Reports. 2019;26(7):1828-40.e4.
79. Stocks CJ, Schembri MA, Sweet MJ, Kapetanovic R. For when bacterial infections persist: Toll-like receptor-inducible direct antimicrobial pathways in macrophages. 2018. p. 35-51.
80. Stocks CJ, Phan M-D, Achard MES, Nhu NTK, Condon ND, Gawthorne JA, *et al*. Uropathogenic employs both evasion and resistance to subvert innate immune-mediated zinc toxicity for dissemination. PNAS. 2019;116(13).
81. Alessandro M, Thekla C, Jenny G, Arnaud P, Norbert R, Oliver G, *et al*. Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. PNAS. 2013;110(19):7820.
82. West AP, Igor EB, Christoph R, Dong Kyun W, Hediye E-B, Paul T, *et al*. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature. 2011;472(7344):476.
83. Baker B, Maitra U, Geng S, Li L. Molecular and cellular mechanisms responsible for cellular stress and low-grade inflammation induced by a super-low dose of endotoxin. J Biol Chem. 2014;289(23):16262-9.
84. Lee JY, Kapur M, Li M, Choi MC, Choi S, Kim HJ, *et al*. MFN1 deacetylation activates adaptive mitochondrial fusion and protects metabolically challenged mitochondria. J Cell Sci. 2014;127(Pt 22):4954-63.
85. Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods. 2017.

86. Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 2014;15(2):R29.
87. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw. 2010;33(1):1-22.
88. Herberg JA, Kaforou M, Wright VJ, Shailes H, Eleftherohorinou H, Hoggart CJ, *et al*. Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children. JAMA. 2016;316(8):835-45.
89. Bermudez LE, Young LS. Tumor necrosis factor, alone or in combination with IL-2, but not IFN-gamma, is associated with macrophage killing of Mycobacterium avium complex. J Immunol. 1988;140(9):3006-13.
90. Roach DR, Bean AG, Demangel C, France MP, Briscoe H, Britton WJ. TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. J Immunol. 2002;168(9):4620-7.
91. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, *et al*. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity. 1995;2(6):561-72.
92. Proietti C, Zakrzewski M, Watkins TS, Berger B, Hasan S, Ratnatunga CN, *et al*. Mining, visualizing and comparing multidimensional biomolecular data using the Genomics Data Miner (GMine) Web-Server. Scientific reports. 2016;6:38178.
93. Griffith DE, Adjemian J, Brown-Elliott BA, Philley JV, Prevots DR, Gaston C, *et al*. Semiquantitative Culture Analysis during Therapy for *Mycobacterium avium* Complex Lung Disease. Am J Respir Crit Care Med. 2015;192(6):754-60.
94. Rosenow T, Oudraad MC, Murray CP, Turkovic L, Kuo W, de Bruijne M, *et al*. PRAGMA-CF. A Quantitative Structural Lung Disease Computed Tomography Outcome in Young Children with Cystic Fibrosis. Am J Respir Crit Care Med. 2015;191(10):1158-65.
95. Kuo W, Andrinopoulou ER, Perez-Rovira A, Ozturk H, de Bruijne M, Tiddens HA. Objective airway artery dimensions compared to CT scoring methods assessing structural cystic fibrosis lung disease. J Cyst Fibros. 2017;16(1):116-23.
96. Kuo W, Ciet P, Tiddens HAWM, Zhang W, Guillerman RP, Straten Mv. Monitoring Cystic Fibrosis Lung Disease by Computed Tomography. Radiation Risk in Perspective. Am J Respir Crit Care Med. 2014;189(11):1328-36.